Growth and productivity assessment of four cowpea genotypes under variable phosphorus levels and soil-moisture conditions

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Declaration

I, Mokoni Kenneth Maduna, declare that there is no one that has ever submitted this dissertation and the results in a fulfillment of a Master of Science in Agriculture degree at University of Mpumalanga, any other University or Higher institutions of learning, with the following title "***Growth and productivity assessment of four cowpea genotypes under variable phosphorus levels and soil-moisture conditions***". I further declare that it is my own work in design and execution under the supervision of Prof F.R. Kutu, Prof M.P. Bopape-Mabapa and Dr S.S. Thosago. All materials and literature from other scholars were rightfully acknowledged.

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Date

Dedication

This research work is dedicated to my beloved family, whose unwavering support and encouragement have been my constant source of strength even when I felt like giving up. To my parents, M.J. Maduna, and late father, A.M. Cossa, for their sacrifices and for instilling in me the values of hard work and perseverance. To my mentors Prof F.R. Kutu and colleagues (Dr. S.S. Thosago, Mr. N. Dladla, and Mr. D. Shabangu), for their guidance and inspiration throughout this journey. And finally, to those who believe in the transformative power of knowledge and research—may this work contribute to a brighter future for all.

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Conferences and Seminars contribution

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Abstract

Cowpea (*Vigna unguiculata* L. Walp) is regrettably observed to have low yields, which are often attributed to, among others, low phosphorus (P) availability and moisture stress conditions. A greenhouse study was initiated to assess the possible effect of integrated soil available P and moisture management strategy in promoting improved growth, productivity, and grain quality attributes of four selected cowpea genotypes. The factorial trial comprised variable P application rates and soil moisture regimes imposed on the four cowpea genotypes as treatment factors. The cowpea genotypes, G (CV17I, CV17F, CV17B, and CV18–1A), P fertilizer levels (0, 30, 60, and 90 kg ha^{-1}) using single super phosphate (8.3% P) applied at planting, and three irrigation regimes (M) were combined to obtain 48 treatment combinations each replicated four times and fitted into a nested design. The soil-moisture levels comprised 15, 50, and 75 kPa water potential imposed at the reproductive stage for 20 days. Growth data were collected 21 days after moisture stress imposition, yield data at harvest, and plant tissue analyses (i.e., mineral, nutritional, and secondary metabolites contents) were essayed after crop harvest. Statistical analysis and mean separation were done using Duncan Multiple Range Test (DMRT) at 5% probability level. The results revealed a differential response of the cowpea genotypes to moisture stress and P levels. A significant ($p \leq 0.05$) GxPxM interaction effect on stomatal conductance (SC), leaf area (LA) and stem diameter (SD), and inconsequential effects were recorded on all measured yield attributes with treatment interaction. The highest recorded SC of 249.92 mmol m^{-2} s^{-1} was from CV17B genotype in adequate soil moisture-filled pot without P addition. Soil moisture stress severity decreased seed yield plant^{-1} by 22 to 37.5% while extreme moisture stress interestingly, had the least effect on a hundred seed weight (12.54 g) in the CV17I genotype. Plant height and chlorophyll content benefitted significantly from P application, with the tallest plants (35.19 cm) obtained at 60 kg P ha^{-1}. Adequate irrigation at 15 kPa significantly increased the number of pods and pod length per plant. The significant GxP interaction effect on the total biological yield and WUE at harvest with CV17I genotype fertilized at 60 kg P ha^{-1} and CV17B without P fertilization attributed to 9.66 g/plant and 1.039 g mm^{-1}, respectively. Furthermore, the results revealed that genotypes and P application exerted a significant ($p \leq 0.05$) effect on the flavonoid, anthocyanin, grain P, Iron (Fe), and zinc (Zn) content with an inconsequential ($p > 0.05$) effect on the total soluble solids and protein content. The second-order GxPxM interaction also exerted a significant ($p \leq 0.01$) effect on all assessed quality parameters except for the protein content. Thus, introducing severe soil moisture stress at 75 kPa significantly reduced flavonoid content by about 10.96% compared to the well-watered regime at 15 kPa, albeit severe soil moisture stress increased the TSS. Likewise, CV18-1A genotype without P addition had cowpea grains with the highest mineral P and Zn content, while CV17I genotype fertilized at 60 kg P ha^{-1} had grains with the highest mineral Fe content. Notably, the principal components (PCs) recorded the highest variability of 32.595%, with the most important traits' loads on PC1 being the number of trifoliate leaves (0.8411), number of branches (0.6539), and chlorophyll content (0.7234). The mean number of seeds per pod displayed a negatively low but significant ($p \leq 0.05$) correlation with the number of pods per plant while showing a very highly significant ($p = 0.000$) and positive correlation with pod length. The flavonoid content had a substantially low and negative association with PC1, PC2, and PC3. Soil moisture effects and P management are important for optimizing cowpea productivity.

Keywords: Biomass accumulation, drought, grain quality, seed yield, smallholder farmers, and water use efficiency.

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CHAPTER 1

General introduction

1.1 Background

Cowpea (*Vigna unguiculata* [L]. Walp) is a protein-rich legume crop mostly cultivated in many parts of the world, especially developing countries, by small-scale farmers for household consumption to meet their socio-economic and nutrition needs (Nkomo *et al.,* 2021; Gerrano *et al.,* 2019). The crop provides nutritive food benefits for both humans and livestock. Cowpeas provide highly nutritious grain with a proximate 23 to 32% protein content, four times higher than most cereal crops (Asiwe and Maimela, 2020). It is valued for its ability to supply fresh leaves, immature pods, and dry grain for resource-poor households. As a result, it increases interest in maximizing the productivity of cowpeas to generate returns in all the harvested parts of the crop. The crop can fix atmospheric nitrogen (N), which has attracted interest amongst smallholder farmers and researchers as an alternative crop in different cropping systems. According to Yahaya (2019), cowpeas can potentially fix up to 337 kg N ha^{-1} in the soil. Santhosh *et al.* (2019) reported that water and nutrients play an important role in ensuring that rhizobia convert atmospheric dinitrogen to usable N form. This improves farmers' production practices since it enables them to reduce the production input costs, such as N fertilizer while improving soil fertility and generating high income due to high grain yield and vegetables. However, most smallholder farmers fail to meet their target of maximizing cowpea productivity through limited natural resources such as poor soil fertility and water supply. Some of the major factors affecting cowpea yield potential in South Africa (SA) include poor soil fertility, drought stress conditions, poor production practices, and the unavailability of improved varieties that can withstand these conditions (Nkomo *et al.,* 2021). The major soil nutrient constraint affecting cowpea production is P availability, which is influenced by numerous factors such as temperature, soil particle size, soil pH, and soil moisture condition. In highly weathered soils, P is reported to be one of the most limiting nutrients for positive crop production (Maranguit *et al.,* 2017; Margalef *et al.,* 2017). Soil P is present as insoluble mineral phosphates; however, most crops do not have easy access to it (Zhu *et al.,* 2018). Legumes depend on naturally occurring P and other nutrients in the soil for their productivity since most smallholder farmers do not apply fertilizer in their fields (Nkaa *et al.,* 2014).

Phosphorus (P) has a significant role in regulating plant respiration, photosynthesis, biosynthesis of membranes, nucleic acids, and other forms of enzymes (Lambers, 2022). Plant nutrients such as P have frequently impacted agricultural production systems, especially crop production, including cowpeas. Most South African soils are inherently low in available P for numerous reasons, including soil degradation due to wind, water erosion, chemical degradation, and deterioration of physical soil properties. Kyei-Boahen *et al.* (2017) reported that 431 kg P ha $^{-1}$ can result in 30% grain yield increase as compared to zero application suggesting that under P deficiency, crop metabolites strains are limited, thereby reducing the concentration of primary and secondary metabolites stimulating growth, development, and defense mechanism against biotic and abiotic stressors.

Drought stress and poor soil fertility conditions have estimated yield loss of 70% on leguminous crops in Africa (Diaz *et al.,* 2017). Among several abiotic factors, drought stress is identified as a major limiting factor affecting cowpea production in Southern Africa (Boukar *et al.,* 2019). Soil moisture is essential to a plant's metabolic activities; hence, the plant can stimulate growth hormones and stress signals during nutrient and water stress (Dada *et al.,* 2021; Khan *et al.,* 2019). Cowpeas is a drought-tolerant crop, but prolonged drought stress can potentially limit its productivity. Previous studies showed that limited soil moisture increases the water use efficiency (WUE) by 6 and 12%, respectively compared to high soil moisture content (Tankari *et al.,* 2019). However, this can vary based on the genotype, environment, and the extent of moisture stress. cowpeas are high in fiber, ashes, carbohydrates, protein, and amino acid contents (Jayathilake *et al.*, 2018). However, the concentration of these essential nutritional attributes can be affected by both biotic and abiotic stress (Boukar *et al.,* 2019; Gerrano *et al.,* 2017). Hence, there is a need to address the knowledge gap in determining the optimum available soil P and appropriate soil moisture level required to guarantee the maximum nutritional quality of cowpeas. Notwithstanding, the unavailability of improved and high-quality cowpea seeds and commercial cowpea production in South Africa, unlike other grain legumes, remains very limited, resulting in low outputs, limited availability, and very high cowpea prices in the SA market. Hence, the yield gap can be bridged through improved cowpea genotypes and the introduction of improved agronomic practices. Despite cowpea having the ability to supply nutritious food, particularly in poor rural households and communities, it remains one of the most neglected crops particularly in South Africa where poverty and hunger at many rural households are still very high.

1.2 Problem statement

The significant continuous increase in the global population calls for an urgent increase in food production to meet the gradually increasing population of about 10 million by 2050 (Van-Dijk *et al.,* 2021). However, most crop production systems still experience drought as one of the major productivity constraints that reduces crop growth and productivity. Future climate change predictions in African countries highlight a possible increase in drought and heat stress that could significantly affect future crop production, including cowpea (Engelbrecht *et al.,* 2024). Despite cowpea being a drought-tolerant crop, growth and yield can be constrained by drought, especially when prolonged. Hence, continuous screening of special genotypic traits for drought and heat tolerance is needed. Similarly, Yahaya *et al.* (2019) reported that screening such crops for improvement in water use efficiency could, therefore, assist in the choice of a superior genotype's adaptability to climate change. On the other hand, deficiencies in soil P mainly occur due to either naturally low P levels in the soil or the depletion of P through continuous cultivation.

Despite the application of P fertilizers to restore soil fertility, about 70 to 90% of the P fertilizers are absorbed and become trapped in various low-solubility soil P compounds without immediate availability for the crop (Balemi and Negisho, 2012). However, there is limited literature on the direct effect of soil P and moisture influence on the mobility and availability of minerals and metabolic response on cowpeas. Research has yet to fully elucidate how varying moisture conditions affect the mineral content in cowpeas, particularly in combination with P application. The effect of soil moisture on the nutritional quality of cowpeas, especially the anthocyanin, flavonoid, and total soluble sugars is not well characterized. Hence, there is a need to substantially examine how moisture stress influences the synthesis of these compounds, especially in varying P levels.

1.3 Rationale

Crops such as cowpeas are well known for their ability to withstand drought and fix atmospheric N in the soil. However, the level at which they can tolerate moisture stress and nutrient stress is still limited. The relationship between cowpeas' moisture regimes and soil P levels is not well studied. Hence, more studies are needed to assess the relationship between the soil moisture, genotypes, and P levels affecting cowpea growth, yield, and nutrient parameters. The desirable gene traits with a good mechanism to tolerate drought and soil P deficiency may vary depending on the genotypes. Huynh *et al.* (2018) reported that several cowpea lines can tolerate drought and heat stress at different growth stages.

Irrigation water use in agriculture has substantially increased crop yield production. As a result, intensive agronomic practices and high-yielding varieties are adopted to maximize production in most developing countries (Velasco-Munoz *et al.,* 2018). Quantifying water use may increase water use efficiency linked to crop production. This will possibly reduce irrigation water utilization and improve the modelling of advanced production systems of managing soil P and moisture without compromising crop productivity. Adequate irrigation frequency and soil fertility management are a primary requirement for sustainable agriculture. Hence, less irrigation and fertilizer inputs use and management is significant. Therefore, improved food production will be met without compromising the limited natural resources and gradual unpredicted climate change.

1.4 Overall Aim and objectives

This study seeks to evaluate an integrated soil available P and moisture management strategy for improving the growth, productivity, and grain quality attributes of the selected four cowpea genotypes. The specific objectives of the study include:

i. To assess the cowpea genotypes' growth, yield, and nutritional parameters under variable soil available P and moisture regimes.
ii. To determine the optimum P rate for growth and productivity of the four cowpea genotypes.
iii. To quantify the effect of variable soil moisture regimes and P fertilizer application on the water use efficiency and mineral and secondary metabolites composition of cowpea genotypes.

1.5 Hypotheses

i. Cowpea genotypes' growth, yield, and nutritional parameters will not differ under variable soil available P and moisture regimes.
ii. The growth and productivity of the four cowpea genotypes under varying P rates and soil moisture regimes will not differ.
iii. Crop water use efficiency and mineral composition of each cowpea genotype will not be quantified under variable soil moisture regimes.

1.6 Dissertation outline

This dissertation consists of six chapters, of which three (Chapter 3, 4, and 5) are constructed and presented as manuscripts to be submitted to journals for publication. The summary of each chapter is as summarized as follows:

Chapter 1: *"Introduction*": This chapter presents foundational and introductory background information on the study overview with a clear outline of the problem statement, the aim, objectives, and hypotheses of the study.

Chapter 2: "*Literature review*": This chapter assessed and reviewed the literature related to the response of cowpeas to Phosphorus application and moisture stress effect on growth, yield, and overall productivity.

Chapter 3: *"Growth and physiological response of four cowpea (Vigna unguiculata Walp L.) genotypes to varying phosphorus rates and soil moisture regimes"*: This chapter examined the growth and physiological response of four cowpea genotypes to varying phosphorus fertilization levels and soil moisture regimes.

Chapter 4: *"Variable phosphorus fertilizer levels and soil moisture regimes affect phenological, yield attributes and Water-use efficiency of four cowpea (Vigna unguiculata L.) genotypes grown under greenhouse conditions"*: This chapter assessed the phenological and yield response of cowpea to P fertilization and moisture deficit conditions while qualifying the Water use efficiency at flowering and harvesting.

Chapter 5: "*Application of variable phosphorus fertilizer rates under different soil moisture conditions affect cowpea grain mineral, protein, and secondary metabolite compositions"*: This chapter examined and quantified the mineral, nutrition, and secondary metabolites of cowpeas genotypes (grain) in response to varying P fertilization rates and moisture regimes.

Chapter 6: "*Conclusion*": This is the final chapter that concludes the study and provides an oversite of the study generally while providing a summary, conclusion, and recommendations.

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CHAPTER 2

Literature review

2.1 Botanical classification, origin and distribution of cowpea

Cowpea *(Vigna unguiculata* (L.) Walp) is a vascular Tracheobionta plant, which belongs to the seed plants super-division Spermatophyte under the Fabaceae family, previously known as Leguminosae, is one of the largest family of flowering plants (Laskar *et al.,* 2019; Farias *et al.,* 2022). In South African local languages cowpea, it is known as *ihlumaya* in isiswati, *akkerboon* or *swartbekboon* in Afrikaans, in Sepedi it is called *dinawa*, in Xitsonga it is *dinaba* or *tinyawa*, in Tshivenda it is *munawa* while in isiZulu it is called *imbumba.* Cowpeas belong to the Faboideae subfamily similarly known as Papilionoideae, which is characterized by flowers with a distinctive butterfly shape (Ogbole *et al.,* 2023). Further, cowpea is classified into several genotypes based on their growth habit, seed size, shape, and colour. Cowpea's originality is not yet well known; however, it is still believed to have originated in Africa (Osipitan *et al.,* 2021). The limited archaeological findings have led to differing perspectives that endorse Africa, Asia, and South America as potential places of origin for cowpeas. However, based on domestication, and wild species diversity of cowpea in Africa, the judgment of its originality is there. Hence, the originality of cowpeas is traced back to West Africa, where it was cultivated for thousands of years (Herniter *et al.,* 2020). Thereafter, cowpeas spread to other parts of Africa and eventually to Asia and the Americas through trade and migration. It is also believed that the southeastern Africa region and the Transvaal region in the Republic of South Africa are the center of diversity of wild *Vigna spp* (Ringo, 2017).

2.2 The importance, uses and nutritional profile of cowpea

Cowpea is a good source of protein and fiber, and it can be used in different dishes (Kyei-Boahen *et al.,* 2017). Cowpea can be used for both human and livestock consumption globally. In most countries in the world, cowpeas are eaten fresh, cooked, or dried. They are often used in soups, stews, and salads. They can also be ground into flour and used to make bread, pasta, and other foods (Naiker *et al.,* 2019). Cowpea leaves can be cooked as "*Morogo*" severed with pap; in other instances where more yield is obtained, the leaves will be cooked, dried, and sold in local communities to generate income. Most commercial farmers cultivate cowpeas for animal feed and as a soil improvement strategy. The crop is capable of fixing N in the soil Pule-Meulenberg *et al.*

(2010) while tolerating drought, making it a significant crop to be cultivated in dry regions while it improves soil fertility. In most African regions, the crop is appreciated for its ability to supply essential human nutrients, such as plant-based protein, fiber, and vitamins (Affrifah *et al.,* 2022). Cowpeas contain 23-32% protein, which is higher than most cereal crops, including animal meat (Abebe and Alemayehu, 2022). Protein is equally significant for building and repairing tissues and is a significant factor in the overall healthy human immune system. This makes them a good crop for dry areas, as most smallholder farmers produce it under dryland farming systems.

2.3 Cowpea production levels

Cowpea is an important crop grown in different South African regions. The main producing provinces in SA are Limpopo, Mpumalanga, Northwest, and KwaZulu-Natal (Asiwe, 2009). However, there is limited centralized data on the production levels of cowpeas, particularly in most African countries, including SA. A report by Abate *et al.* (2012) stipulates an increase in cowpea production in most African countries. Similarly, Gerrano *et al.* (2019) and Kamara *et al.* (2018) reported that the largest producers of cowpeas in SA are small-scale farmers under dryland farming with an estimated grain yield of less than 0.025 – 0.3 t ha $^{-1}$. As a result, there are minimal records regarding the size of the area under production. In 2019, the estimated average yield of cowpea production in SA was 4848 tonnes Gerrano *et al.* (2022), which is less compared to the production of 2012 – 2014. However, this estimate is based on data from a limited number of farmers, and the actual production level is likely higher. Asiwe (2009) reported that in SA cowpea production ranged between 0.25 and 1.0 t ha^{-1}, which is very low compared to the US in 2019 production of 11,750 tons, with an estimate of 2.25 t ha^{-1} (Osipitan *et al.,* 2021).

The major constraints contributing to low cowpea production are diseases and insect pests, low soil fertility, prolonged drought stress, and lack of quality seed and improved varieties (Asiwe, 2022; Kebede and Bekeko, 2020). Hence there are several initiatives underway to improve the production of cowpeas in SA, including the development of new genotypes that are resistant to both abiotic and biotic conditions such as poor soil fertility low in P, pests, diseases, and prolonged drought stress (Gerrano *et al.,* 2022; Aremu *et al.,* 2017). Nkomo *et al.* (2021) estimated that the world cowpea production is 6.2 MMT, with Africa accounting for 96.7% of the total production and being led by the top five cowpea-producing countries in the world FAOSTAT (2021), which are Nigeria, Ghana, Niger, Burkina Faso, and Mali account for over 70% of the world's cowpea production (Beshir *et al.,* 2019). Nigeria is reported to be the largest producer of cowpeas in the world, with an estimated 2.5 million tons of cowpeas globally, as it is used as food and animal feed (Asiwe, 2022; Kebede and Bekeko, 2020). Niger is the second-largest producer of cowpeas in the world with an estimated 1.4 million tonnes of the total world's cowpeas production (Boukar *et al.,* 2019). Similarly, Kebede and Bekeko (2020); Boukar *et al.* (2019) reported that Burkina Faso is the third-largest producer of cowpeas. Moreover, Omomowo and Babalola (2021) estimated 554 286 tonnes of cowpeas for Burkina Faso in 2016. Additionally, other major contributors with significant cowpea production include Ghana, Mali, Cameroon, Senegal, and Sudan.

2.4 Major constraints affecting cowpea production

Despite cowpea's potential to withstand harsh conditions and supply food to most vulnerable households, the crop still experiences a lot of challenges attributed to different factors such as biotic and abiotic stress (Mwale *et al.,* 2017; Olasupo *et al.,* 2016). As a result, most smallholder farmers in most African countries still receive less than the potential yield of 2500 kg ha^{-1} (Molosiwa and Makwala, 2020). Some of the major constraints include:

2.4.1 Biotic stress

Biotic stressors are the adverse effects on plants caused by living organisms such as weeds, pests, and pathogens (Gupta *et al.,* 2021; Gull *et al.,* 2019). These factors can impose significant damage to crops leading to yield reduction and, as a result, ultimate economic loss for farmers and causing food shortages for consumers. Despite these challenges, many plants including cowpeas have evolved various defense mechanisms to combat some of these biotic stresses. Physical and chemical defenses and induced resistance are some of the mechanisms used by plants to combat these stresses (Rajput *et al.,* 2021; Jalil and Ansari, 2019; Amorim *et al.,* 2018). The plant's physical defense includes the plant's outer cuticle layer, trichomes, and thorns that prevent insects from feeding on the plant (Mostafa *et al.,* 2022; Chaudhary *et al.,* 2018). The defense mechanism improved crop adaptability and minimal yield reduction despite the challenges. Chemical defenses include secondary metabolites production, such as alkaloids, terpenes, and phenolics that discourage herbivores and pathogens (Divekar *et al.,* 2022; Sánchez-Sánchez and Morquecho-Contreras, 2017). Induced resistance involves the plant's ability to activate mechanisms of defense in response to biotic stress signals. However, some cowpea genotypes are susceptible to several fungal diseases, such as anthracnose, rust, and powdery mildew (Omomowo and Babalola, 2021). These diseases can cause leaf spots, stem rot, and pod blight, leading to yield reduction and poor crop seed quality. Moreover, bacterial diseases such as bacterial blight and bacterial pustule can cause wilting of leaves, stem rot, and seed discoloration. Despite biotic stress challenges in cowpea production worldwide, using integrated strategies and disease-resistant varieties can help minimize the impact.

2.4.2 Abiotic stress

The non-living environmental factors affecting the growth and productivity of plants are described by Gull *et al.* (2019) as abiotic stressors. The abiotic stressors include high temperature, drought stress, salinity, heavy metals, and radiation (Zhang *et al.,* 2022). These factors impose a substantial reduction in cowpea production worldwide, causing significant losses in yield and resulting in poor seed quality (Singh *et al.*, 2022; Yasir *et al.,* 2021). The most common form of abiotic stress in plants is drought, which occurs when soil moisture levels are inadequate to meet the water demands of plants (Tron *et al.,* 2015). Drought stress can reduce some of the plants' metabolic activities. Plant develops several mechanisms to tolerate drought stress (Omomowo and Babalola, 2021). Amongst these mechanisms assumed by plants include dehydration avoidance, drought escape, and drought tolerance. According to Santos *et al.* (2018) drought causes various changes in photosynthetic metabolism and stomatal closure. Stomatal closure is the first response of most plants to drought stress as a result of preventing water loss from transpiration paths (Pirasteh-Anosheh *et al.,* 2016). Despite cowpeas being drought tolerant, prolonged drought can reduce their growth rates (Carvalho *et al.,* 2019). Hence, there is a need to identify genes involved in stress response pathways and use genetic engineering techniques to introduce improved genes to cowpeas. Approaches include breeding for stress tolerance traits and improving agronomic practices such as irrigation and soil fertility management.

Salinity stress is also a major abiotic stress that affects cowpea production. Yasir *et al.* (2021) reported that regardless of cowpeas being able to use an alternative crop for salt-affected soils, salinity still severely inhibits shoot and root length, the number of branches, leaf relative water content, chlorophyll, and biomass per plant in lentils. Salinity stress transpires when soil and/or water contains high salt levels, which interferes with nutrient uptake and plant growth. Chourasia *et al.* (2021) reported that salinity leads to ion toxicity, osmotic stress, and oxidative damage. Salinity stress occurs once the intensity of salt in the soil goes beyond the tolerance limit of the plant. Salinity triggers a harmful effect on the rate of seed germination, the growth of seedlings, the length of roots and shoots, the production of dry matter, and the vigor index of cowpea plants

(Maamallan *et al.*, 2021). Despite that Singh *et al.* (2022) highlighted that many plants develop different strategies to cope with salinity stress, including ion exclusion, ion compartmentalization, and the production of compatible solutes such as glycine betaine, which was supported by Li *et al.* (2022) who reported that acclimation of root growth is critical survival of the plant during salinity stress, moreover roots of cowpea contain different developmental and physiological adjustments to salt stress through managing plant growth, provision of new pathways to improve salt tolerance of plants using root adjustment strategies (Kebede *et al.,* 2020). Hence, this suggests that salinity leads to decreased root water uptake, ion toxicity, and decreased plant growth and yield. An increase in research interest and outputs in developing genotypes that are more tolerant to abiotic stress is needed.

2.5 Cowpea phenotypic and genetic diversity for improved and sustainable production

Genetic diversity among cowpea plants can be classified as genotype variation. Genetic diversity is assessed by physiological and phenotypic traits, including the plant height, pod length, and days before flowering and molecular makeup (Nkhoma *et al.,* 2020). However, Kameswara (2004) reported that molecular makeup is still limited in classifying quantitative traits as influenced by moisture conditions and possible fertility. Cowpea genotype diversity is important; it provides a gene pool that can be used to develop new genotypes with adaptive characteristics, such as genes for drought tolerance and pest and disease resistance while retaining high yields (Mafakheri *et al.,* 2017). Genotype variation also contributes to the long-term sustainability of cowpea production. A greater genetic variety increases the likelihood of obtaining improved agronomic traits as well as suitable genes for improvement (Gupta and Salgotra, 2022; Sinha *et al.,* 2021). Plant breeders can benefit most from improved cowpea genotype diversity since it makes selecting suitable parents for pollination easier and reduces ineffective crosses. A large genetic foundation helps to create superior genotypes with the highest yield and finest grain quality, whereas a small genetic base makes plants more vulnerable to many pressures (Eltaher *et al.,* 2021; Zheng *et al.,* 2021). In order to assess genotypes for their diversity among the accessions for desirable parental choices, it is critical to characterize cowpea accessions (Gouda *et al.,* 2020; Nkhoma *et al.,* 2020). Selecting the best genotype is a crucial production decision that can help minimize the risk of crop failure. This choice is influenced by multiple factors, such as yield potential, agronomic traits, regional conditions, and the genotype's adaptability (Nascimento *et al.,* 2023; Ren *et al.,* 2022). The capacity to thrive in water-stressed conditions is a fundamental factor in selecting the appropriate genotype, as choosing the inappropriate one can significantly limit yield (Azrai *et al.,* 2022; Ahmed *et al.,* 2020). Improved genotypes will yield higher regardless of limited water conditions.

Nkhoma *et al.* (2020) evaluated the phenotypical traits from two localities in Zambia, assessing the genomic diversity amongst 100 different cowpea genotypes germplasm collected from Southern Africa. The study found a relatively high level of genetic diversity among the genotypes evaluated. Hence, the long-term viability of cowpea production depends on the preservation of genetic diversity. Preserving and protecting the genetic diversity of the crop will advance the development of cowpea genotypes that are adaptable to changing environmental conditions and resistant to pests and diseases (Nkomo *et al.,* 2019; Mafakheri *et al.,* 2017). This will make it more likely that cowpeas will continue to be a significant food crop for many years.

2.6 Soil fertility and mobilization for effective cowpea Phosphorus Use Efficiency (PUE)

Cowpea has the ability to fix atmospheric N through a symbiotic relationship with N-fixing bacteria called rhizobia Mahmud *et al.* (2020), with the potential to fix up to 337 kg N. ha^{-1} in the soil. However, cowpea still requires adequate amounts of Phosphorus (P), Nitrogen (N), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulfur (S), and other micronutrients for optimal growth and yield. Anago *et al.* (2021) reported that improving N, P, K, and CEC in the topsoil increases cowpea grain yields, using N as a starter boast. Moreover, Hiama *et al.* (2019) revealed that the application of 60 kg P ha^{-1} improves cowpea N fixation and K uptake. Cowpea prefers soils with good organic matter content, which can improve soil structure, water-holding capacity, and nutrient availability. Phosphorus is an essential nutrient for plant growth and is often a limiting factor in crop production, including cowpeas. Despite limited literature on the quantity required for optimizing productivity, Cowpea requires adequate P for proper growth and development. However, excessive use of P fertilizers can lead to environmental pollution (Siddque *et al.,* 2023; Ashitha *et al.,* 2021). Making the concept of PUE a critical component in sustainable agronomic practices, which can be described as the ability of plants to use P efficiently for growth, development, and productivity. Fageria *et al.* (2013) describe PUE as an important index in determining the use of the applied fertilizer by crop. Phosphorus use efficiency can be improved through various management practices such as balanced fertilization, the use of P-solubilizing microorganisms, crop rotation, and conservation tillage. As a result, plant breeding can also play a crucial role in improving PUE by selecting more efficient genotypes for acquiring and using P. Moreover, approaches such as improving the root system's ability to acquire P from the soil can result in increased root surface area through root branching or by increasing the production of root hairs that are involved in nutrient uptake (Heuer *et al.,* 2017).

Alkama *et al.* (2009) research revealed that cowpea genotypes differ in their PUE, with some genotypes showing higher PUE than others. Furthermore, Kugblenu *et al.* (2014) found that some cowpea genotypes had higher grain yield and PUE than others under low-P conditions. These suggest that selecting cowpea genotypes with high PUE could be a favorable approach to improving cowpea productivity in low-P soils. Applying P fertilizer at the right time and rate can enhance cowpea growth and yield while minimizing P losses to the environment. Mensah *et al.* (2017) and Ayodele and Oso (2014) demonstrated that applying P fertilizer at planting significantly enhances early vegetative growth and P uptake and produces the highest number of nodules, flowers, and pods in cowpea compared to late application in the season or not applying it at all. Additionally, fertilizer application and other management practices, such as intercropping legumes or using organic amendments like compost or manure, can also improve cowpea PUE by enhancing soil fertility and nutrient availability (Paramesh *et al.,* 2023; Batyrbek *et al.,* 2022). Latati *et al.* (2014) showed that cowpea-maize intercropping improves soil P availability, uptake, and cowpea yield compared to sole cropping, which is significantly associated with the changes in the rhizosphere of cowpea. Hence, improving PUE not only has environmental benefits but also economic benefits for farmers. By reducing the amount of fertilizer needed to achieve optimal yields, farmers can save on input costs while maintaining or even increasing crop yields. Therefore, improving PUE is an important goal in sustainable agriculture.

2.7 Cowpea water requirement, water use, and deficit tolerance for optimal productivity

Cowpea requires adequate soil moisture for good germination and growth; however, it can tolerate drought conditions once fully established. The recommended irrigation schedule for cowpea is once every 7-10 days, depending on the soil type and climatic conditions (Salim *et al.,* 2018). Cowpea has a relatively low water requirement compared to other legumes. The amount of water cowpeas require depends on different factors, including the growth stage, soil type, temperature, and humidity. A report by Mrazova *et al.* (2017) detailed that legumes require about 350-450 mm of water during the growing season. Effective irrigation scheduling, which considers factors such as crop growth stage, soil type, climate, and available water resources, is crucial for ensuring that cowpea receives the approximately 350-450 mm of water they need per growing season, as indicated by (Mrazova *et al.,* 2017).

However, this can differ depending on the region, type of legumes, and the specific genotype. Once cowpea is exposed to water deficit conditions, the rooting system becomes shallow, making it prone to prolonged stress (Mohammed *et al.,* 2022; Tatsumi *et al.,* 2019). Therefore, it is important to provide adequate water during the early stages of growth to ensure good establishment and during the flowering stage to optimize yield. Shahzad *et al.* (2021) outlined that phenological growth stages, such as flower and pod initiation, are substantially dependent on soil moisture availability. Barros *et al.* (2020) reported that an increase in temperature leads to an increase in water demand, leading to greater losses of moisture through evapotranspiration in agricultural production systems. As a result, water availability for plants will be reduced. Cowpea can tolerate some degree of moisture stress without significant yield loss. However, prolonged drought or waterlogging can cause severe yield losses. This suggests that when soil moisture drops below 50% field capacity, irrigation should be applied. As Farouk and Amany, (2012) reported that the largest reduction of cowpea grain yield was observed under severe water stress at 30% field capacity. However, there is still a research gap on cowpeas' irrigation frequency since moisture can affect overall crop productivity (Alves *et al.,* 2020; Zhang *et al.,* 2019). Hence, this might suggest that yield may not be the only functionality of the amount of water applied but time of application and frequency.

2.8 Assessment of plant's drought tolerance mechanism

Plant sensitivity to water stress significantly reduces plant growth and development (Osakabe *et al.,* 2014). However, plants have different responsive mechanisms that assist them to tolerate water stress and adapt to water-limited conditions. To overcome drought stress conditions, plants have evolved various morphological, physiological, biochemical, cellular, and molecular mechanisms (Fang and Xiong, 2015). Moreover, the drought resistance mechanisms plants adopt include drought avoidance, escape, and tolerance (Bashir *et al.,* 2021). Plants under drought stress can modify their physiological and morphological traits to adjust to the conditions. This may include decreasing transpiration, stomatal closing, reduced leaf area, senescence acceleration, and increasing root system to absorb water and nutrients (Bashir *et al.,* 2021; Seleiman *et al.,* 2021). According to Poudel *et al.* (2021) stress tolerance, susceptibility, and yield stability index are key indicators used to assess a plant's ability to withstand drought conditions. Seleiman *et al.* (2021)

reported that a greater value of the tolerance index suggests that plants are more sensitive to water stress.

The major indicators of drought tolerance in crops primarily encompass physiological parameters linked to osmotic adjustment (OA), which is a crucial factor in drought resistance (Bashir *et al.*, 2021; Abid *et al.,* 2018). The osmotic adjustment potential resulting from the net accumulation of solutes in response to water stress, helping plants maintain turgor pressure (Blum, 2017). Zegaoui *et al.* (2017) stated that the increase in proline content of cowpeas after water stress has also been associated with the mechanisms of drought tolerance. Drought avoidance in plants is their ability to maintain adequate water levels or cellular hydration under drought-stress conditions (Gupta *et al.*, 2020). The strategies for drought avoidance vary widely and largely depend on the intensity of stress occurring at the different parts of the plant (Zia *et al.*, 2021). In general, plants mitigate dehydration during drought by closing their stomata to regulate water loss from leaves, thereby minimizing the transpiring surface while sustaining root water absorption as the soil dries (Bandurska *et al.,* 2022; Zia *et al.*, 2021). Furthermore, Jung *et al.* (2019) observed that plants with greater root density and deeper rooting systems demonstrate improved water uptake, making this an essential mechanism for coping with drought stress. Consequently, crops with deep root systems are advantageous in environments where water is accessible in deeper soil layers. Seleiman *et al.* (2021) explained that plants employ drought avoidance by boosting water absorption, limiting water loss, or improving internal water retention to prevent tissue desiccation.

Plants can escape drought by completing their life cycle before major water stress occurs (Shavrukov *et al.*, 2017; Basu *et al.*, 2016). Plants with drought escape traits will germinate from dormant seeds only when there is enough water (Bhatt *et al.,* 2022). Subsequently, they survive with a limited water supply to terminate vegetative growth while inducing reproduction quickly to shorten the life cycle, thus resulting in early maturity. The effect of water stress differs among genotypes, as some genotypes are highly tolerant (Shao *et al.,* 2008). Moreover, Shao *et al.* (2008) reported that genetic constitutions are better adapted to water stress and have a higher relative water content, leaf area index, rate of photosynthesis, and transpiration rate. Hayatu *et al.* (2014) findings are supported by Tankari *et al.* (2019) concluding that water stress gradually reduced CO_2 assimilation rates due to the decrease of stomatal conductance.

2.9 Soil moisture deficit and variable P condition on growth, N fixation, and productivity attributes of cowpea

The level and duration of the moisture stress affect plants' response to their survival. Such responses are characterized as complex, and more research is needed to explain all of them properly. A comprehension of the selection and breeding of drought-tolerant crops depends on crop responses to water stress. Plant growth and developmental stages differ based on the level at which the plant is, this can include seed emergence/germination, vegetative growth stage, flowering, fruit set, and full physiological maturity. Growth and development in plants are achieved through the process of cell division expansion and differentiation, which Mahajan *et al.* (2018) reported greatly depends on moisture availability. Thus, moisture stress reduces cell division, expansion, and differentiation, concluding the observed effect of which is reduced plant growth. One of the physiological processes most susceptible to moisture stress is cell expansion, which is brought on by a reduction in turgor pressure. When the water deficit is significant, the water flow from the xylem to the surrounding elongating cells is disrupted, which inhibits cell growth. According to Wijewardana *et al.* (2019) soybean plants, stem length drastically decreases when there is a water shortage. The expansion of leaf area, which is primarily influenced by leaf turgor, temperature, and assimilate supply, is another growth characteristic that a water deficit impacts (Santos *et al.,* 2020; Win and Oo, 2015). Optimal leaf development is crucial for photosynthesis and dry matter output.

Crops under water stress reduce yield significantly, similar to cowpeas (Ntombela, 2012). In developing countries, drought is portrayed as a constant to agricultural productivity (Meza *et al.,* 2021). As a result, most countries still experience extremely low cowpea yield due to this moisture constraint than the achievable yield in cowpea (Omomowo and Babalola, 2021; Yahaya *et al.,* 2019). Kyei-Boahen *et al.* (2017) reported that P deficiency and moisture stress can reduce pod and seed production in cowpeas, despite excessive P and soil moisture application can negatively affect cowpea growth and yield. Adusei *et al.* (2020) results have shown that the combined effect of P and moisture on cowpea growth and yield is complex and varies depending on the soil fertility and genotype similar observation is reported by Chtouki *et al.* (2022) on chickpeas. For example, under low-moisture conditions, P application can enhance cowpea yield by increasing pod number per plant and seed weight (Nkaa *et al.,* 2014). Comparably, moisture stress during the early growth stages of cowpeas can reduce pod number per plant but increase seed weight (Dadson *et al.,* 2005).

Cowpeas require adequate moisture for optimal growth and yield, but moisture stress or excessive moisture can also have negative effects.

2.10 The response of stomatal density and conductance adaptation

Stomata are microscopic pores on the leaf surface that regulate gas exchange and water loss. The density and conductance of stomata are vital parameters that determine a plant's ability to cope with water stress. Research has shown that chickpea plants significantly decrease the stomatal conductance under moderate and severe water stress conditions to minimize water loss through transpiration Chtouki *et al.* (2022), which is a research adaptive strategy by plants for maintaining water balance under drought conditions (Yang *et al.,* 2021). However, it is reported that P availability in the soil plays a significant role in modulating stomatal density and conductance. As a result, the P and moisture effect interaction has a critical role in the stomatal conductance and density. Furthermore, Chtouki *et al.* (2022) reported that under adequate soil moisture conditions with P application increased stomatal density by approximately 12%, while a 7% increase is observed under moderate water stress. Revealing that when plants have adequate moisture, and P tends to allow more moisture to be lost through evaporation. As a result, more water is lost than utilized, compared to where less moisture is available; the plant will use the minimal available resources and try to store them for later use compared to the accessible ones.

Pirasteh-Anosheh *et al.* (2016) reported that, under severe moisture stress, stomatal density is affected and stomata completely close, which is closely dependent on plant species, so tolerant species control the status of their stomata to allow carbon fixation and photosynthesis as well as improving their WUE. The plant's ability to maintain gas exchange under less severe stress is less effective under extreme conditions. Additionally, Kashiwagi *et al.* (2015) highlighted that plants present various morphological and physiological adaptations under nutrient and moisture stress conditions to enhance their ability to effectively absorb and utilize water and mineral resources. This adjustment allows the plant to survive despite water and nutrient deficit conditions. A previous study by Meier *et al.* (2021) reported on the interaction effect between water and P was mainly focused on the impact of P rate applied or P concentration in the soil being a proposed solution on crop growth and development.

2.11 Stomatal regulation and photosynthetic adaptation of plants under water deficit conditions

Stomatal regulation is a key process in maintaining photosynthetic capacity under drought stress. The primary response of most plants to severe drought is stomatal closure, which prevents water loss via transpiration and reduces CO_2 uptake, decreasing photosynthesis (Pamungkas and Farid, 2022). This response is driven by direct water evaporation from guard cells without metabolic action. Stomatal closure during drought helps plants avoid dehydration, a vital survival strategy. Stomatal conductance is closely related to midday vapor pressure deficit, as observed in olive trees (*Olea europaea* L.) (Rodriguez-Dominguez *et al.,* 2019). Lower stomatal conductance in the afternoon compared to the morning further emphasizes the plant's strategy to conserve water during peak transpiration periods (Brito *et al.,* 2018). Reduced stomatal conductance directly impacts the photosynthesis rate, as observed in soybean under drought stress (Wang *et al.,* 2018). This reduction is primarily due to stomatal limitations and secondarily to metabolic impairments.

The balance between stomatal and non-stomatal factors in photosynthetic inhibition varies among plant species. In olives, stomatal conductance limits photosynthesis under mild or moderate drought, while non-stomatal factors play a role under severe stress (Rodriguez-Dominguez *et al.,* 2019). Drought stress influences stomatal morphology, including size, density, and distribution changes. These morphological traits significantly affect gas exchange and water use efficiency. Under drought conditions, some plants exhibit increased stomatal density but reduced size and aperture, enhancing water use efficiency (Bertolino *et al.,* 2019). This adaptation allows plants to maintain gas exchange while minimizing water loss.

The relationship between stomatal density and drought stress varies among plant species. For example, in almonds (Prunus dulcis), drought stress does not significantly affect stomatal length, width, or density (Yadollahi *et al.,* 2011). In contrast, maize exhibits increased stomatal density under reduced soil water content (Nguyen *et al.,* 2022). These interspecific differences highlight the diverse strategies employed by plants to cope with water stress. Khan *et al.* (2023) highlighted that P deficiency can significantly affect the opening and closure of stomata in various plant species, including wheat and sugar beet. This finding outlines that deficiency impairs photosynthesis and growth and disrupts water regulation processes. Adequate P application assists plants in maintaining efficient stomatal function, thereby enhancing their ability to withstand the environmental stresses of drought.

2.12 Effect of soil moisture and phosphorus on chlorophyll content and photosynthetic efficiency

Chlorophyll is an essential physiological component of plants and plays a key role in growth and development through the process of photosynthesis. Soil moisture effect results in changes in photosynthetic pigments and components of plants (Verma *et al.,* 2020). Which damages photosynthetic mechanisms and reduces Calvin cycle enzyme activities, which are significant for crop productivity. Plant growth can be influenced by various physiological and biochemical functions of chlorophyll synthesis, photosynthesis, metabolic nutrients, ion uptake and translocation, and respiration, which are highly influenced by water deficit. Severe water deficit situations can decrease chlorophyll, protein, and soluble sugar levels, which have also been reported in plants under drought stress (Gurrieri *et al.,* 2020; Du *et al.,* 2020).

The chlorophyll content is a critical indicator of photosynthetic capacity and is significantly influenced by P, soil moisture, and plant species type. A study by Chtouki *et al.* (2022) on chickpea leaves reveals that P supply enhances chlorophyll content across all irrigation regimes, with adequate moisture conditions (75% FC), Poly-P and Ortho-P fertilizers increasing the chlorophyll content by 42% and 23%, respectively, compared to unfertilized treatments. they further outlined that in moderate and severe water stress (50% and 25% FC), P fertilization results in a 25% and 14% increase in chlorophyll content, respectively (Chtouki *et al.,* 2022). Indicating that P application mitigates the adverse effects of water stress on chlorophyll synthesis and retention. Similarly, the chlorophyll content of chickpea leaves was significantly increased with P supply under all irrigation regimes, with no significant difference between P fertilizer forms. However, a remarkable decrease in chlorophyll content was observed under drought stress conditions (Chtouki *et al.,* 2022). Research consistently shows that drought stress can decrease chlorophyll content due to reduced photosynthetic activity and accelerated chlorophyll degradation (Khayatnezhad and Gholamin, 2021; Sarani *et al.,* 2014).

2.13 Effect of moisture stress and soil P application on the mineral composition of cowpea

Phosphorus, Zinc, and Iron are among the most limiting macro- and micronutrients in human diets, contributing to widespread global deficiencies. Zinc is essential for pregnant women and children due to its vital role in immune system function and infection prevention. A severe deficiency during pregnancy can result in complications during childbirth, low birth weight, impaired child growth, and decreased resistance to infectious diseases. According to Bird and Eskin (2021), P presents vital functions in skeletal and non-skeletal tissues and is pivotal for energy production. At the same time, iron is primarily involved in transferring oxygen from the lungs to tissues. It plays a significant role in metabolism as a component of some proteins and enzymes (Abbaspour *et al.,* 2014). Cowpea, being a crucial crop in most sub-Saharan African regions, provides sustenance richness, particularly protein, iron, and zinc from its leaves, pods, and seeds.

Improving the availability of essential nutrients in human diets, especially iron and zinc, in developing countries is a global priority. Additionally, Tóth *et al.* (2021) reported that sub-optimal concentrations of Fe and Zn in crops and wheat grain cause micronutrient deficiencies in humans. Hence, enhancing the accessibility of essential nutrients in food can be achieved through improved agronomic practices such as optimal use of micronutrient-rich fertilizers and genetic enhancement of high-quality varieties. The application of major nutrients like NPK fertilizers without considering micronutrient needs can lead to lower levels of micronutrients in the edible parts of crops. This is because the micronutrient content of grains primarily depends on the supply of soil nutrients and fertilizers containing micronutrients. Approaches such as improving agronomic practices including phosphate fertilizer application, and the development of high P use efficient crop varieties, would assist in achieving high-quality cowpea yields on soils with low available P. Genotypes with improved P efficiencies use various mechanisms, including increased soil P solubilization and mobilization, improved root traits, and enhanced P acquisition and accumulation in edible tissues. Phosphorus application, especially at high rates, could have adverse effects if it diminishes zinc availability, as seen in certain cereal crops (Ayeni *et al.,* 2018).

Soil moisture negatively impacts mineral absorption and distribution during the critical grain initiation stage, impairing seed and grain quality. Despite the high mineral concentration in leguminous crops like cowpeas, poor soil fertility and nutrient deficiency can negatively impact grain mineral content (Tóth *et al.,* 2021). Nutrient movement, such as P in plants and grains, depends on moisture levels, affecting grain quality. Which can also interrupt the mineral flow in the soil, causing an imbalance in plant nutrition. Hence, adequate nutrient availability in plants reveals that moisture stress is due to plants' poor absorption and transpiration flow. Soil moisture stress reduces potassium, calcium, iron, P, and zinc in legume grain quality according to Sarkar *et al.* (2021), leading to poor seed quality. Moreover, Seleiman *et al.* (2021) reported that mineral content reduction could be caused by weakened transpiration, stomatal conductance, and decreased root function under drought conditions. Moisture deficit stress affects internal plant processes, which disrupts many cellular and whole plant functions, negatively affecting plant growth and reproduction (Kapoor *et al.*, 2020). As such, the implication will affect the crop yield quality. Moisture stress can inhibit the transportation of minerals such as Fe, Mg, Zn, and Cu to seeds (Wijewardana *et al.,* 2019). Sustainable solutions are essential to increase grain yields with a balanced mineral composition to enhance food security.

2.14 Effect of Phosphorus and soil moisture condition on nutrition composition of cowpea seeds

2.14.1 Protein

Proteins are classified as primary metabolites, playing an imperative role in the growth, repair, maintenance, and regeneration of tissues in living organisms (Pereira, 2018). Cowpea is regarded as one of the highest-quality plant protein sources, with grain containing approximately 28% protein (Gerrano *et al.*, 2019). Making this protein a vital component for human nutrition and a significant source of income through sales. However, soil moisture stress, especially during the initiation stage, has been shown to decrease the protein content (Seleiman *et al.*, 2021; Abid *et al.*, 2018). This is because drought conditions often reduce carbohydrate accumulation more than nitrogen Rakszegi *et al.* (2019), resulting in higher protein concentrations in the grain (Flagella *et al.*, 2010). However, this increase in protein content can come at the expense of yield, as water stress typically reduces overall grain size and weight. Conversely, adequate soil moisture generally supports improved grain yields but may lead to lower protein content due to a dilution effect, where higher yields result in lower protein concentrations (Walsh *et al.,* 2020). The availability of moisture enhances the uptake and assimilation of nitrogen, but if the nitrogen is not adequately supplied, the protein content may still be low (Zayed *et al.*, 2023).

The buildup of insoluble phosphates and chemical fixation in soil reduces P consumption ability in plants (Everest *et al.,* 2022). Adequate P is essential for root development, efficient water uptake, and other nutrients, including nitrogen. Adequate P fertilization enhances nitrogen use efficiency, which is directly related to protein synthesis in grains; P application can increase protein content by improving nitrogen uptake (Duncan *et al.,* 2018). Since nitrogen is a key component of proteins, its efficient use, supported by adequate P, can lead to higher protein content. However, excessive P without corresponding nitrogen levels might not increase protein content significantly. Soils deficient in P often show reduced protein content, as plants struggle with poor root development and nutrient uptake. Conversely, soils with optimal P levels support better plant health, improving grain quality and protein content.

2.14.2 Total soluble sugars

Total soluble sugars (TSS) are one of the plant metabolites which significantly influence grain quality. Total soluble sugars are essential for the energy requirements of developing plants and contribute to the taste, nutritional value, shelf life, and overall sensory acceptability of foods (Neela and Fanta, 2019). Soil moisture directly impacts TSS accumulation in grains by influencing physiological processes such as photosynthesis and nutrient uptake. Adequate soil moisture levels promote efficient photosynthesis and the translocation of photosynthates to develop in plants. However, Sibomana *et al.* (2015) reported lower TSS content in tomatoes due to well and moderate water conditions. Contrarily, drought conditions can increase TSS content as a protective response to osmotic stress (Yu *et al.*, 2020). However, extreme drought stress may ultimately lead to reduced TSS due to inhibited photosynthesis and reduced carbohydrate availability. Furthermore, P availability plays a significant role in energy transfer, photosynthesis, and carbohydrate metabolism, all of which are directly linked to TSS content in grains.

Optimum P availability can potentially enhance TSS accumulation by promoting root development, improving water and nutrient uptake, and enhancing photosynthetic efficiency. However, Meena *et al.* (2021) reported that proline and total soluble sugars increased under low P, drought, and combined stresses in mungbean. Conversely, under drought conditions, the effectiveness of P application on TSS accumulation may be diminished due to restricted nutrient uptake. These intricate relationships between soil moisture, P availability, and TSS accumulation underscore the importance of considering these factors to optimize grain quality.

2.14.3 Flavonoids

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables, and grains (Panche *et al.,* 2016). Soil moisture plays a critical role in plant metabolism and can impact the synthesis of flavonoids. Studies have shown that water stress caused by drought can increase the flavonoid concentration in plants (Park *et al.*, 2023; Yang *et al.*, 2020). This can be attributed to a defense mechanism, as flavonoids help protect plants from drought-induced oxidative stress.

On the other hand, optimal moisture conditions are essential for balanced growth and flavonoid synthesis (Wang *et al.,* 2014). While moderate water stress can enhance flavonoid content, severe stress may reduce grain yield and potentially lower flavonoid content per grain unit.

Furthermore, P availability in the soil has been found to directly influence the metabolic pathways involved in flavonoid production (Kayoumu *et al.,* 2023; Shah and Smith, 2020). However, Chea *et al.* (2021) reported that excessive P applications beyond 90 kg ha^{-1} can led to nutrient imbalances, ultimately reducing flavonoid concentrations. Additionally, balanced P application is crucial for maximizing both yield and flavonoid content. Optimal P can mitigate some negative effects of drought on flavonoid synthesis by ensuring that the plant's energy requirements are met during water stress, while well-managed soil moisture can improve P uptake, further enhancing flavonoid synthesis (Seleiman *et al.,* 2021; Wang *et al.,* 2013).

2.14.4 Anthocyanins

Anthocyanins are a group of flavonoid compounds responsible for the red, purple, and blue colors in many fruits, vegetables, and grains. Beyond their role in pigmentation, anthocyanins are valued for their antioxidant properties, contributing to the health benefits of consuming anthocyanin-rich foods (Mattioli *et al.,* 2020). However, different agronomic practices, including soil moisture and P management strategies, play crucial roles in determining the anthocyanin content in grains. As a result, water deficit conditions have been reported to increase the synthesis of anthocyanins as part of plants' stress response potentially protecting plant tissues from oxidative damage (Shi *et al.,* 2023).

Contrarily Liu *et al.* (2022) P deficiency can reduce anthocyanin content due to impaired plant growth and metabolism. Conversely, optimal P levels can enhance anthocyanin production by ensuring the availability of ATP and other substrates required for biosynthesis. The timing and method of P application also play a significant role in influencing anthocyanin accumulation in grains. These findings suggest that integrated management practices considering both soil moisture and P availability are essential for maximizing anthocyanin content in grain crops.

2.15 Conclusion

The literature above outlines the relationship between P application and soil moisture conditions on cowpea growth, yield, mineral content, and nutritional composition, which is critical yet underexplored. The existing literature provides insights into how P and soil moisture independently affect cowpeas, but significant gaps remain, particularly concerning their combined effects and the specific mechanisms involved. Despite P being essential for cowpea growth and yield, it influences root development, energy transfer, and photosynthesis. However, the optimum levels of P maximize growth and yield without causing a negative effect on crop quality, especially in varying soil moisture conditions. Research overlooked how P availability interacts with soil moisture conditions. Phosphorus influences the uptake and translocation of essential minerals like Fe and Zn in cowpeas. While studies have shown that adequate P levels can enhance Fe and Zn content, the precise pathways through which P affects these minerals and how soil moisture modulates these effects are poorly understood.

The effect of P on secondary metabolites such as anthocyanins and flavonoids is less documented. Limited research suggests that P may enhance these compounds due to its role in plant metabolism, but the extent to which this is affected by varying soil moisture is unclear. The response of cowpeas to P under different moisture regimes needs further exploration, especially in terms of how moisture stress alters P efficiency. Soil moisture influences the mobility and availability of minerals like Fe, Zn, and P. Research has yet to fully elucidate how varying moisture conditions affect the mineral content in cowpeas, particularly in combination with P application. The effect of soil moisture on the nutritional quality of cowpeas, especially in terms of anthocyanin, flavonoids, total soluble sugars, and protein content, is not well characterized. Studies need to examine how moisture stress influences the synthesis of these compounds, particularly in the context of varying P levels.

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CHAPTER 3

Growth and physiological response of four cowpea (*Vigna unguiculata* Walp L.) genotypes to phosphorus and soil moisture regimes

Abstract

Soil moisture and fertility constraints are key constraints that limit crop productivity in most African soils. The study assessed the growth and physiological response of the cowpea genotypes to variable P fertilization rates and moisture regimes. To achieve this objective, a factorial trial fitted into a nested, completely randomized design (CRD) was established under a controlled environment, comprising four cowpea genotypes (G), four phosphorus (P) levels, and three irrigation (M) regimes imposed at the onset of the reproductive stage for 20 days. Growth and physiological data were collected 21 days after moisture stress imposition. Results obtained revealed a significant ($p \leq 0.05$) GxP and PxM interaction effect on leaf area, with the highest leaf area (1068.4 m^2) obtained from CV17B genotype fertilized at 90 kg P ha^{-1} rate. Genotype and PxM interaction significantly ($p \leq 0.05$) influenced the measured plant growth parameters, resulting in the CV18-1A genotype producing a remarkably tallest (152 cm) plant and highest (33.36 µmol m^2) chlorophyll content. A three-way interaction of Genotypes x P fertilization x Moisture regimes significantly ($p \leq 0.05$) affected the stomatal conductance. The CV18-1A genotype fertilized at 90 kg P ha^{-1} under severe moisture stress experienced reduced stomatal conductance to 96.38 mmol m^{-2} s^{-1}. In contrast, the CV17B genotype without P application under a well-watered regime recorded the highest (249.92 mmol m^{-2} s^{-1}) stomatal conductance. This study affirms the importance of soil moisture condition and P fertilization on cowpea growth and physiological attributes with CV17B and CV18-1A genotypes identified as potential candidates that can thrive better under limited moisture and P deficit conditions.

Keywords: yield, drought, soil fertility

3.1 Introduction

Cowpea (*Vigna unguiculata Walp* L.) is one of the oldest indigenous crops known in most African countries, whose domestication is historically in line with other staple African crops such as pearl millet and sorghum. Despite the uncertainty about their center of origin, cowpeas have been widely recognized for their adaptability to different soil conditions, tolerating infertile acid soils, and being less resilient to cold conditions (Mekonnen *et al.*, 2022). Optimal growth and productivity occur in well-drained soils while it can still yield well in water-limited and heavy soil conditions (Iizumi *et al.*, 2024). Soil moisture is a critical limiting factor in crop production across African countries, particularly in South Africa, where high temperatures and low humidity exacerbate uneven rainfall and high evapotranspiration rates (Johnston *et al.*, 2016). These unfavorable climatic conditions pose significant challenges to agricultural production and food security, highlighting the need to explore resilient crops. Cowpea, known for its drought tolerance, is a potential crop to achieve critical poverty reduction goals, improved nutrition, and ecosystem resilience. Despite their resilience, cowpea still faces serious productivity threats under prolonged water stress and high temperatures typical of tropical semi-arid and arid regions. These stressors are often studied separately and have a complex interaction effect that can significantly impact plant growth and productivity (Chtouki *et al.*, 2022). Hence, climate change increases global temperatures and alters rainfall patterns thus exacerbating crop production challenges. Nonetheless, cowpea represents a valuable grain and fodder crop cultivated in tropical and subtropical regions.

Poor soil fertility and soil moisture deficit can induce plant stress beyond the threshold level thereby causing irreversible damage to plant physiology and metabolic pathways (Ahluwalia *et al.*, 2021; Ahmad *et al.*, 2022). Notwithstanding its production potential and nutritional benefits, cowpea remains an under-researched and underutilized crop with many available genotypes highly vulnerable to drought particularly during the reproductive growth stage. Water scarcity continues to threaten crop production in sub-Saharan Africa (Leal Filho *et al.*, 2022). Adequate water in the root zone of crops is essential for vital physiological processes such as germination, transpiration, and nutrient absorption (Nguyen *et al.*, 2017), leading to decreased photosynthates and stomatal closure, and reduced carbon dioxide assimilation and photosynthetic efficiency. Earlier studies have shown that water stress reduces plant height, leaf number, and dry matter production in various crops, including cowpeas (Olorunwa *et al.*, 2023; Jayawardhane *et al.*, 2022). It also disrupts assimilates partitioning and enzyme activities that are essential for grain productivity thus reducing grain yield and quality (Sehgal *et al.* 2018). The negative effects of sequential leaf loss and leaf area having a consequential effect on grain yield have also been reported (Zhu *et al.*, 2020).

Phosphorus is crucial in early root formation, seed development, crop yield quality, and different biochemical processes such as photosynthesis, respiration, energy storage and transfer, cell division, and elongation (Khan *et al.,* 2023). Phosphorus deficiency is regarded as a major constraint globally in crop production and worse in tropical Africa. Most tropical African soils are inherently low in available P due to the high presence of P-fixing aluminium and iron oxides that limit P availability to plants (Tauro *et al.,* 2023; Poswa, 2016). Despite cowpeas' ability to fix a significant amount of N in soil under sufficient P, the crop still struggles to access P in the soil due to P fixation (Mardamootoo *et al.,* 2021). Recent studies by Li *et al.* (2022; 2021) suggest that low P stress inhibits N-fixing enzyme activity in legume nodules thus reducing the amount of N fixed in the soil. Similarly, other numerous research works have also shown that soils with low available P levels and/or high P-fixing properties require the use of P-formulated fertilizers as a quick and effective solution for soil improvement (Yang *et al.,* 2022; Ros *et al.,* 2020). Regrettably, the situation is exacerbated by the non- to sub-optimal use of synthetic P fertilizers by most South African smallholder farmers, which is largely attributed to the high cost and, sometimes, their unavailability in rural markets. Kugblenu *et al.* (2014) reported that cowpea genotypes with high P accumulation under P deficiency and drought stress can be found within the genetic resources of improved breeding materials, albeit limited.

Cowpea production through traditional farming practices promotes adaptive resistance of genotypes edaphoclimatic in local communities. Moreover, cowpea genotypes are genetically diverse, inculcating explorable genes in breeding programs. In South Africa, numerous research has been carried out on various cowpea genotypes over the past three decades, including on landraces. However, research on screening for special growth and physiological traits is still limited to improving special breeding traits. Hence, this study examined the response of four cowpea genotypes to soil P and moisture stress conditions to propose a strategy for improving its productivity in rural communities not only to diversify the food base but also as a crop rotation strategy for soil improvement, specifically N fixation (Li *et al.,* 2022).

3.2 Methodology

3.2.1 Study sites

A pot experiment was conducted under a greenhouse condition at the University of Mpumalanga (UMP) farm (25°26' 8"S, 30°58′51"E; 719 meters above sea level, masl) at the Mbombela campus during 2023/24-summer planting season. The greenhouse's temperatures and humidity control systems occasionally witnessed breakdown due to the wet wall and fan malfunctioning, sometimes reaching 35-39°C and 58-72% humidity during hot days. However, during the day, temperature ranged between 18 to 29°C, which is the normal temperature for most commercial greenhouses (Shamshiri *et al.,* 2018). To mitigate the potential confounding effect of occasional spikes in daily temperatures on the crop, the greenhouse door was frequently opened to allow for free air movement and limit heat buildup in the greenhouse and the manually filling of the water reservoir for the greenhouse wet wall to promote the cooling. The area records an average annual temperature of 18.3°C and an average annual precipitation of about 934 mm (Kwata *et al.,* 2018; Mangani *et al.,* 2019). Soil for the experiment was collected from a local farmer's field involved in cowpea production at Barberton (25°48' 05.6"S, 30°56′34.5"E) where there was no recent history of planting and/or fertilizer application. The available P level in the soil was considered adequate but not optimal as it was 16 mg kg^{-1}, which, according to FERTASA (2016), soils with available P at 15 to 18 mg kg^{-1} are considered adequate for soybeans. The P content in the soil was established through standard laboratory soil analysis.

3.2.2 Plant material and soil collection

Four-grain cowpea genotypes, namely, CV17I, CV17F, CV17B, and CV18–1A, were used in this study. Seeds were obtained from seed multiplication and evaluation research projects at UMP involving different smallholder farmers' fields. These cowpea genotypes were selected based on the growth patterns and yield potential such as fodder and grain. However, these genotypes have an indeterminate growing habit. Surface soil at 0 to 30 cm depth was collected within 2 m × 2 m area of uncultivated plots. Collected soil samples were taken to the Agricultural Research Council Tropical and Subtropical Crops (ARC-TSC), Nelspruit laboratory for detailed analysis of the Physio-chemical properties of the soil, including phosphorus (P), nitrogen (N), and potassium (K), electrical conductivity (EC), soil pH and soil particles analysis.

3.2.3 Experimental design, setup, and treatments application

A factorial trial fitted into a nested completely randomised design (CRD) was established under a controlled environment, comprising four cowpea genotypes (G), four phosphorus (P) levels, and three irrigation (M) regimes adding up to 48 sub-treatments which were replicated four (4) times resulting in 192 experimental units. A single super phosphate fertilizer (8.3% P) was used as P factor (F1) with four rates applied at planting, four cowpea genotypes (F2) and F3 being the variable moisture regimes outlined in Table 3.1, respectively. The treatment arrangement for one replication is outlined in Table 3.2 with the various treatment combinations.

Table 3.1: Treatment factors and their meaning

Genotypes	**Moisture regimes (**kPa)	**Phosphorus levels (**kg P ha^{-1}**)**
G1 – CV17I	M1 – 15	P0 – 0
G2 – CV17F	M2 – 50	P1 – 30
G3 – CV17B	M3 – 75	P2 – 60
G4 – CV18-1A		P3 – 90

Table 3.2: Arrangements of single and integrated treatments

M1			**M2**			**M3**		
G1xP1	G1xP2	G1xP3	G1xP1	G1xP2	G1xP3	G1xP1	G1xP2	G1xP3
G3xP1	G3xP2	G3xP3	G3xP1	G3xP2	G3xP3	G3xP1	G3xP2	G3xP3
G2xP1	G2xP2	G2xP3	G2xP1	G2xP2	G2xP3	G2xP1	G2xP2	G2xP3
G4xP1	G4xP2	G4xP3	G4xP1	G4xP2	G4xP3	G4xP1	G4xP2	G4xP3

The soil for the trial was collected from the surface 0 – 30 cm depth and allowed to air dry. The soil was subsequently passed through 5 mm sieve to remove roots, stones, and pebbles; and 10 kg weighed into each 30 cm plastic pot. Thereafter, the weighed soils in plastic pots were steamsterilized at 250°C for 2 hours to achieve contaminants-free (i.e., pathogen, pests, weed seeds etc.) planting soil. The soil was allowed to cool down for four days before planting. Three to four seeds were planted in each pot at a depth of 20 mm and regularly irrigated to 90% field capacity at 15 kPa, monitored using tensiometers and the addition of 250 ml water. The seedlings were thinned to two vigorously growing seedlings in each pot at two weeks after planting (WAP).

Moisture stress treatment was imposed at the reproductive growth stage, representing 7 WAP for 20 days, which is one of the physiological stages that, when affected by moisture stress, reduces the overall crop productivity; moreover, a similar imposition was based on a study by Coka (2024). This was induced by withdrawing water application to varying degrees to achieve a desired soil water potential based on the treatment. Treatment with the well-watered regime continued receiving adequate water once when the upper limit of 15 kPa was reached to ensure the plants were adequately irrigated without any moisture stress. The moderate water stress regime treatment received irrigation once a week with 150 ml tap water in addition to the upper tensiometer limit of 50 kPa while maintaining a lower limit of 20 kPa. The severe stress regime was maintained at an upper limit of 75 kPa, with a reduced irrigation volume of 100 ml once after nine days, specifically when the tensiometer reading exceeds 75 kPa, respectively.

3.2.4 Data collection

3.2.4.1 Growth and Physiological Data

Growth and physiological data were collected 21 days after moisture-stress imposition at 75% physiological maturity growth stage which included the number of leaves, number of dropped leaves, and number of branches were counted manually from each plant. The plant height, leaf width, and length were measured using a measuring tape (cm), while the stem diameter was measured using a vernier calliper (mm). The stomatal conductance (mmol^{-2} s^{-1}) and chlorophyll content (µmol m^{-2}) were measured between 09:00 and 13:00 using an SC-1 leaf porometer and CCM-200 Plus chlorophyll content meter, respectively, collected by placing the instrument on the leaf surface after proper calibration. The leaf area (LA) was computed using the following formula as reported by Osei-Yeboah *et al.* (1983) and cited by Ekeleme and Nwofia (2005):

$$LA\ (cm^2) = L \times W \times 2.235 \qquad \textit{Equation 3.1}$$

Where: L = leaf length, W = leaf width, and 2.235 = k-coefficient

3.3 Statistical analysis

The collected data was subjected to analysis of variance (ANOVA) using GenStart statistical software version 24.1 and Duncan Multiple Rage Test (DMRT) was used for mean separation at the probability level of 5%. To predict the effect of Genotypes, P levels, and soil moisture regimes on the measured variable a statistical model analysis was used (Equation 3.2). Data on phenology and yield responses to the different P rates were modelled using the quadratic polynomial equation (Equation 3.3). The quadratic model used was the best fit for the data to determine the optimal P rate for the measured parameters. The best-fit line for the quadratic model was performed using Microsoft excel® 2013. Pearson correlation coefficients were computed to examine the degree of association among the measured traits. Data were also subjected to principal component and cluster analyses using PAleontological STatistics (PAST) statistical software version 4.03. The principal component analysis (PCA) was performed as a standardized data technique as described by Mendonça *et al.* (2018) to obtain the eigenvalues from the characteristic roots of covariance and the eigenvectors from the vector elements. Cluster analysis was performed following the chord distance coefficient while the average-linkage method of the datasets as described by Saidaiah *et al.* (2021). To predict the effect of P levels and soil moisture regimes on the measured variables, the following statistical model was used for the analysis of variance:

$$Y_{ijk} = \mu + P_i + M_j + G_k \ (P \times M)_{ijk\ldots\ldots\ldots\ldots} + E_{ijk} \qquad \textit{Equation 3.2}$$

Where: Y_{ijk} = measured parameters, μ = population mean, P_i = P levels effect, M_j = Moisture effect, G_k = Genotype $(P \times M)_{ijk}$ = the interactive effect of P levels and moisture regimes, and E_{ijk} = random error effect

Quadratic polynomial equation:

$$Y = a + b1X + b2X^2 \qquad \textit{Equation 3.3}$$

Where: Y represents the dependent variable (parameters); ‘a’ is the intercept; ‘b’ represents the slope of the line, and ‘X’ is the optimum P rate.

3.4 Results

3.4.1 Soil samples analysis results

The results of the soil sample analysis for the study are detailed and presented in Table 3.3. The measured pH (KCl) value of 5.94 was recorded, indicating that the soil used was slightly acidic. The available P measured in the soil is 16 mg kg^{-1}, nitrate 33.20 mg kg^{-1} while 3.90 mg kg^{-1} of ammonium was recorded (Table 3.3). The exchangeable Ca, Mg, K, and Na contents were 1600, 340, 113, and 13 mg kg^{-1}, respectively (Table 3.3). The sand, silt, and clay contents of the soil were 64%, 13%, and 23%, respectively indicating sandy loam textural class.

Table 3.3: Results of the physico-chemical characteristics of the soil used for the study

Parameter	**Value**
pH (KCl) 1:2.5	5.94
milligram/kilogram (mg/kg)	
Nitrate (N-NO_3)	33.20
Ammonium (N-NH_4)	3.90
Phosphorus (P (Bray 1))	16
Potassium (K)	113
Calcium (Ca)	1600
Magnesium (Mg)	340
Sodium (Na)	13
Iron (Fe)	5.80
Copper (Cu)	1.12
Zinc (Zn)	7.64
Manganese (Mn)	60.40
Sulfate (S-(SO_4))	17
***Sulfur Value (S-value)**	**11.156**
% Sand	64
% Silt	13
% Clay	23

3.4.2 Results of *p*-values for the measured parameters

Table 3.4 provides details of the obtained *p*-values for all measured parameters following ANOVA. Cowpea genotypes, moisture regimes, and the GxM interaction significantly ($p \leq 0.05$) affected the mean number of trifoliate leaves and stomatal conductance. Similarly, the variation in cowpea genotypes, soil moisture regimes, and the GxP interaction significantly ($p \leq 0.05$) affected cowpea leaf length. The variation in cowpea genotypes exerted a significant ($p \leq 0.05$) effect on the number of leaves dropped while the variation in soil moisture regimes and cowpea genotypes exerted significant ($p \leq 0.05$) effect on the chlorophyll content and mean number of branches of cowpea plants. In contrast, none of the variation in P levels, and GxP, GxM and PxM interactions as well as the GxPxM interaction exerted any significant ($p \geq 0.05$) effect on the mean number of branches. The variation in cowpea genotypes and PxM interaction exerted a significant ($p \leq 0.05$) effect on the measured plant height. However, P application levels, moisture regimes variation as well as the GxP and GxM interactions had inconsequential ($p \geq 0.05$) effect on plant height. Cowpea genotypes and moisture regimes individually, and the GxP and PxM interaction exerted a significant ($p \leq 0.05$) effect on the leaf length, while none of the treatments applied exhibited any significant ($p \geq 0.05$) effect on the leaf width. Furthermore, the PxM interaction exerted a significant ($p \leq 0.05$) effect on the cowpea leaf area and plant stem diameter. Interestingly, the variation in cowpea genotypes and moisture regimes significantly ($p \leq 0.05$) affected the chlorophyll content, while variation in cowpea genotypes, moisture regimes, and GxM exhibited a significant ($p \leq 0.05$) effect on stomatal conductance. Among all measured variables, only the stomata conductance exhibited a significant ($p \leq 0.05$) response to the second-order interaction (GxPxM) effect.

Table 3.4: *p*-values for growth and physiological attributes of cowpea

S of V	**PH**	**LL**	**LW**	**LA**	**SD**	**CCl**	**SC**	**NoFLP**	**NoFDL**	**NofBs**
G	0.000***	0.014*	0.472^{ns}	0.302^{ns}	0.817^{ns}	0.000***	0.001***	0.000***	0.000***	0.000***
P	0.372^{ns}	0.088^{ns}	0.605^{ns}	0.220^{ns}	0.942^{ns}	0.5041^{ns}	0.803^{ns}	0.098^{ns}	0.142^{ns}	0.440^{ns}
M	0.059^{ns}	0.0376*	0.355^{ns}	0.053 ^{ns}	0.327^{ns}	0.000***	0.000***	0.005**	0.446^{ns}	0.028*
GxP	0.534^{ns}	0.009**	0.601^{ns}	0.054 ^{ns}	0.701^{ns}	0.986^{ns}	0.764^{ns}	0.727^{ns}	0.655^{ns}	0.257^{ns}
GxM	0.244^{ns}	0.675^{ns}	0.845^{ns}	0.801^{ns}	0.771^{ns}	0.115^{ns}	0.000***	0.009**	0.231^{ns}	0.822^{ns}
PxM	**0.022****	0.013*	0.081^{ns}	0.042*	0.002**	0.311^{ns}	0.144^{ns}	0.643^{ns}	0.129^{ns}	0.865^{ns}
GxPxM	0.541^{ns}	0.7670^{ns}	0.422^{ns}	0.438^{ns}	0.117^{ns}	0.787^{ns}	0.000***	0.469^{ns}	0.547^{ns}	0.167^{ns}

S of V implies sources of variation; G= Genotypes, P = Phosphorus levels, M= Moisture regimes, ns= not significant; *, ** and *** indicate significant effect of treatment at 5%, 1% and 0.1% level, respectively; NoFLP = Number of leaves per plant, NoFDL = Number of dropped leaves, NofBs = Number of Branches, PH = Plant Height (cm), LL = Leaf Length (cm), LW = Leaf Width (cm), LA = Leaf Area (cm^{2}), SD = Stem Diameter (mm), CCl = Chlorophyll Content (µmol m^{-2}), SC = Stomatal conductance (mmol^{-2} s^{-1}).

3.4.3 Main treatment effects on measured growth and physiological attributes of grain cowpea

The results reveal that the CV18-1A genotype had the greater leafing ability, the tallest plant height, and the highest chlorophyll content among all the tested genotypes (Table 3.5). Similarly, CV17I genotype dropped more leaves while having a greater branching ability. Interestingly CV17B genotype had a statistically higher leaf length and stomatal conductance of 22.38 cm and 170.61 mmol m^{-2} s^{-1} respectively (Table 3.5). Despite the inconsequential variation in the leaf area CV17B genotype had 15% wider leaf area as compared to CV17F genotype. The P application rates on the measured parameters had a relative less than >50% coefficient of variation range between 17.93 to 44.11% (Table 3.5). Notwithstanding the non-significant difference in plant height for CV17I and CV18-1A, the latter is 8.1% taller. Contrary to the P application on number of dropped leaves and chlorophyll content with 64.55 and 57.80% coefficient of variation respectively (Table 3.5). Equally, well-watered regime resulted in a greater number of trifoliate leaves, number of branches, chlorophyll content and stomatal conductance. While the imposition of 50 kPa moisture regime only improved the leaf length and leaf areal. Remarkably Table 3.5 shows that moisture regimes imposed an inconsequential variation on the number of dropped leaves of about 13% difference between the 15 and 50 kPa moisture regime with a relatively high coefficient of variation of 64.55%.

3.4.4 Treatment interaction effect of measured growth and physiological attributes of grain cowpea

The effect of interaction between phosphorus application and moisture regimes on the measured growth and physiological attributes is presented in Table 3.6. The results revealed that cowpea plants without P fertilizer addition in this soil but with adequate moisture at 15 kPa irrigation gave the highest mean number of trifoliate leaves and highest chlorophyll content. The mean plant height measured from P0xM1, P1xM1 and P3xM3 interaction treatments representing 115.56, 116.08 and 112.66 cm, respectively are statistically comparable with moderate variation (i.e., *CV* of 21.67%). Interestingly, the results also reveal that 60 kg P ha^{-1} application under severe moisture stress (75 kPa) significantly increased the stem diameter by 11% compared to the similar P rate with moderate moisture regime (50 kPa) although with a relatively low variation (i.e., *CV* of 18.69%).

Table 3.5: Single factor effect genotypes, P levels and Moisture regimes on growth and physiological attributes

Treatment factors	**PH**	**LL**	**LW**	**LA**	**SD**	**CCl**	**SC**	**NoFLP**	**NoFDL**	**NofBs**
Genotypes										
CV17I	104.64^{b}	21.42^{ab}	14.41^{a}	702.54^{a}	4.72^{a}	25.39^{b}	164.75^{a}	9.98^{b}	4.38^{a}	3.73^{a}
CV17F	84.75^{c}	20.56^{ab}	13.75^{a}	657.49^{a}	4.57^{a}	25.29^{b}	151.22^{ab}	7.08^{c}	2.54^{bc}	2.42^{c}
CV17B	76.86^{c}	22.38^{a}	14.92^{a}	775.57^{a}	4.58^{a}	16.23^{c}	170.61^{a}	6.58^{c}	2.98^{b}	2.15^{c}
CV18-1A	152.92^{a}	19.67^{b}	15.23^{a}	690.50^{a}	4.60^{a}	33.36^{a}	140.54^{b}	11.35^{a}	1.92^{c}	3.21^{b}
! CV	21.67	20.00	33.61	44.11	18.69	57.80	24.05	17.93	64.55	26.21
Phosphorus levels (kg/ha)										
0	105.54^{a}	20.95^{a}	14.80^{a}	700.43^{a}	4.65^{a}	25.45^{a}	158.67^{a}	9.19^{a}	3.08^{a}	2.85^{a}
30	100.03^{a}	20.61^{a}	13.77^{a}	649.07^{a}	4.56^{a}	22.38^{a}	160.16^{a}	8.65^{a}	3.42^{a}	2.75^{a}
60	105.65^{a}	20.18^{a}	14.70^{a}	695.37^{a}	4.65^{a}	26.59^{a}	153.62^{a}	8.39^{a}	2.73^{a}	2.89^{a}
90	107.94^{a}	22.28^{a}	15.03^{a}	781.22^{a}	4.61^{a}	25.86^{a}	154.67^{a}	8.77^{a}	2.58^{a}	3.00^{a}
! CV	21.67	20.00	33.61	44.11	18.69	57.80	24.05	17.93	64.55	26.21
Moisture regimes (kPa)										
15	110.33^{a}	19.96^{b}	13.87^{a}	631.20^{a}	4.49^{a}	31.76^{a}	184.57^{a}	9.19^{a}	3.14^{a}	3.08^{a}
50	102.68^{a}	21.86^{a}	15.05^{a}	761.25^{ab}	4.63^{a}	25.40^{b}	153.54^{b}	8.79^{ab}	2.72^{a}	2.73^{b}
75	101.36^{a}	21.20^{ab}	14.81^{a}	727.12^{b}	4.73^{a}	18.05^{c}	132.23^{c}	8.27^{b}	3.00^{a}	2.81^{ab}
! CV	21.67	20.00	33.61	44.11	18.69	57.80	24.05	17.93	64.55	26.21

! CV = Coefficient of variation, NoFLP = Number of leaves per plant, NoFDL = Number of dropped leaves, NofBs = Number of Branches, PH = Plant Height (cm), LL = Leaf Length (cm), LW = Leaf Width (cm), LA = Leaf Area (cm^{2}*), SD = Stem Diameter (mm), CCl = Chlorophyll Content (µmol m*$^{-2}$*), SC = Stomatal conductance (mmol*$^{-2}$ *s*$^{-1}$*).*

Table 3.6: Phosphorus and moisture regime interaction effect on measured growth and physiological attributes

Treatment factors	PH	LL	SD	CCl	SC	NoFLP
P0xM1	115.56^{a}	18.84^{b}	4.38^{ab}	36.15^{a}	184.59^{ab}	9.81^{a}
P0xM2	100.04^{ab}	24.18^{a}	4.98^{ab}	23.28^{abc}	150.74^{bcd}	9.44^{ab}
P0xM3	101.04^{ab}	19.83^{ab}	4.59^{ab}	16.92^{bc}	140.67^{cd}	8.31^{ab}
P1xM1	116.08^{a}	20.53^{ab}	4.59^{ab}	31.73^{ab}	182.08^{abc}	9.31^{ab}
P1xM2	97.86^{ab}	20.69^{ab}	4.44^{ab}	21.52^{abc}	171.95^{abc}	8.69^{ab}
P1xM3	86.16^{b}	20.61^{ab}	4.64^{ab}	13.90^{c}	126.45^{d}	7.94^{b}
P2xM1	101.77^{ab}	20.04^{ab}	4.71^{ab}	29.79^{abc}	194.74^{a}	8.69^{ab}
P2xM2	109.59^{ab}	18.81^{b}	4.06^{b}	31.36^{ab}	145.48^{bcd}	8.38^{ab}
P2xM3	105.58^{ab}	21.69^{ab}	5.19^{a}	18.62^{bc}	120.63^{d}	8.13^{ab}
P3xM1	107.92^{ab}	20.43^{ab}	4.31^{ab}	29.36^{abc}	176.89^{abc}	8.94^{ab}
P3xM2	103.25^{ab}	23.734^{a}	5.04^{ab}	25.44^{abc}	145.98^{bcd}	8.69^{ab}
P3xM3	112.66^{a}	22.67^{ab}	4.48^{ab}	22.78^{abc}	141.15^{bcd}	8.69^{ab}
! *CV*	21.67	20.00	18.69	57.80	24.05	17.93

! CV = Coefficient of variation, PxM implies treatment interaction at variable P rates and moisture (M) levels; NoFLP = Number of leaves per plant, PH = Plant Height (cm), LL = Leaf Length (cm), SD = Stem Diameter (mm), CCl = Chlorophyll Content (µmol m^{-2}) and SC = stomatal conductance (mmol^{-2} s^{-1}).

The genotype x phosphorus interaction revealed that CV17B genotype fertilized at 90 kg P ha^{-1} gave the longest leaf length and highest leaf area (Figure 3.1). Similarly, the genotype x moisture level interaction showed that CV18-1A genotype irrigated at 15 kPa produced a significantly highest number of trifoliate leaves per plant while CV17B genotype also irrigated at 15 kPa produced the highest stomatal conductance (Figure 3.2). Furthermore, in the second order interaction (GxPxM), CV17B genotype irrigated at 15 kPa and without P fertilization gave the highest mean stomata conductance value of 249.92 mmol m^{-2} s^{-1} (Figure 3.3).

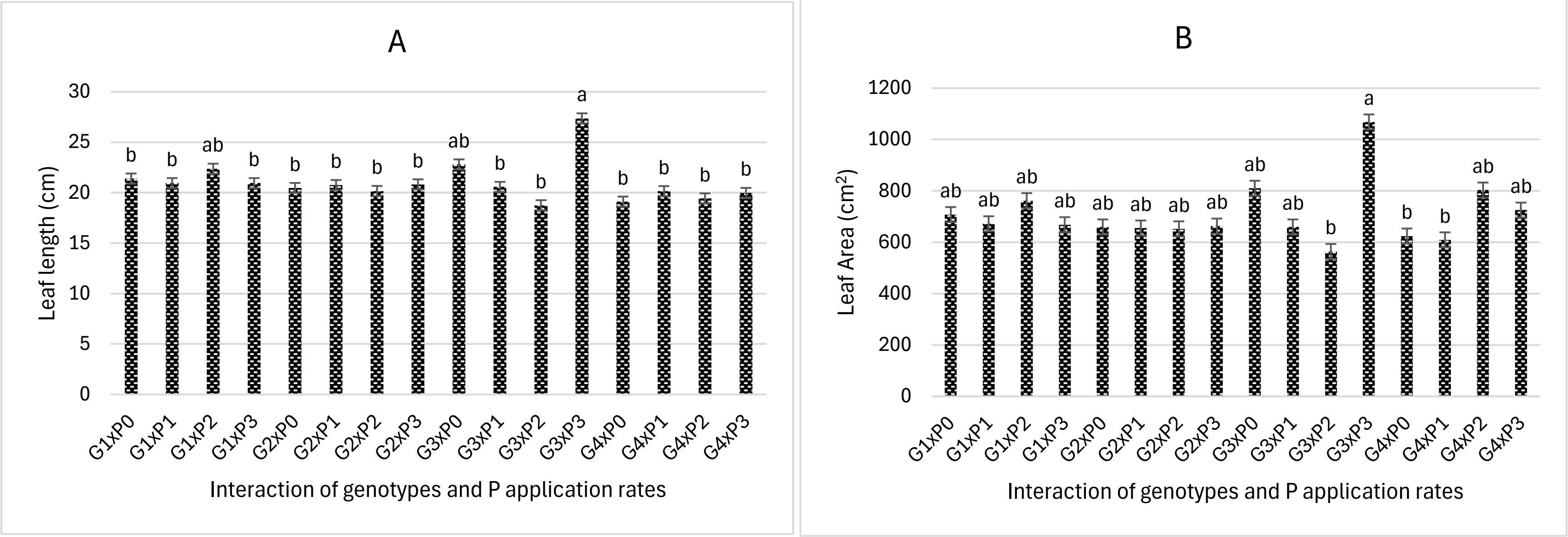


Figure 3.1: Genotypes x P application interaction effect on cowpea leaf length (A) and leaf area (B).

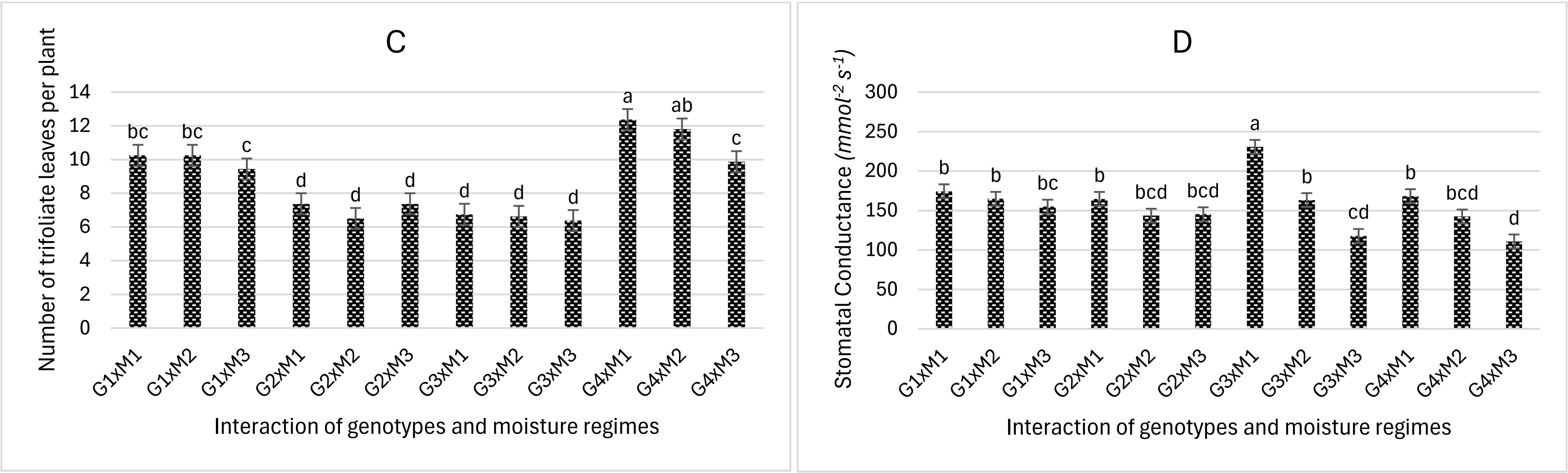


Figure 3.2: Genotypes x moisture regime interaction effect on cowpea leaf length (C) and leaf area (D).

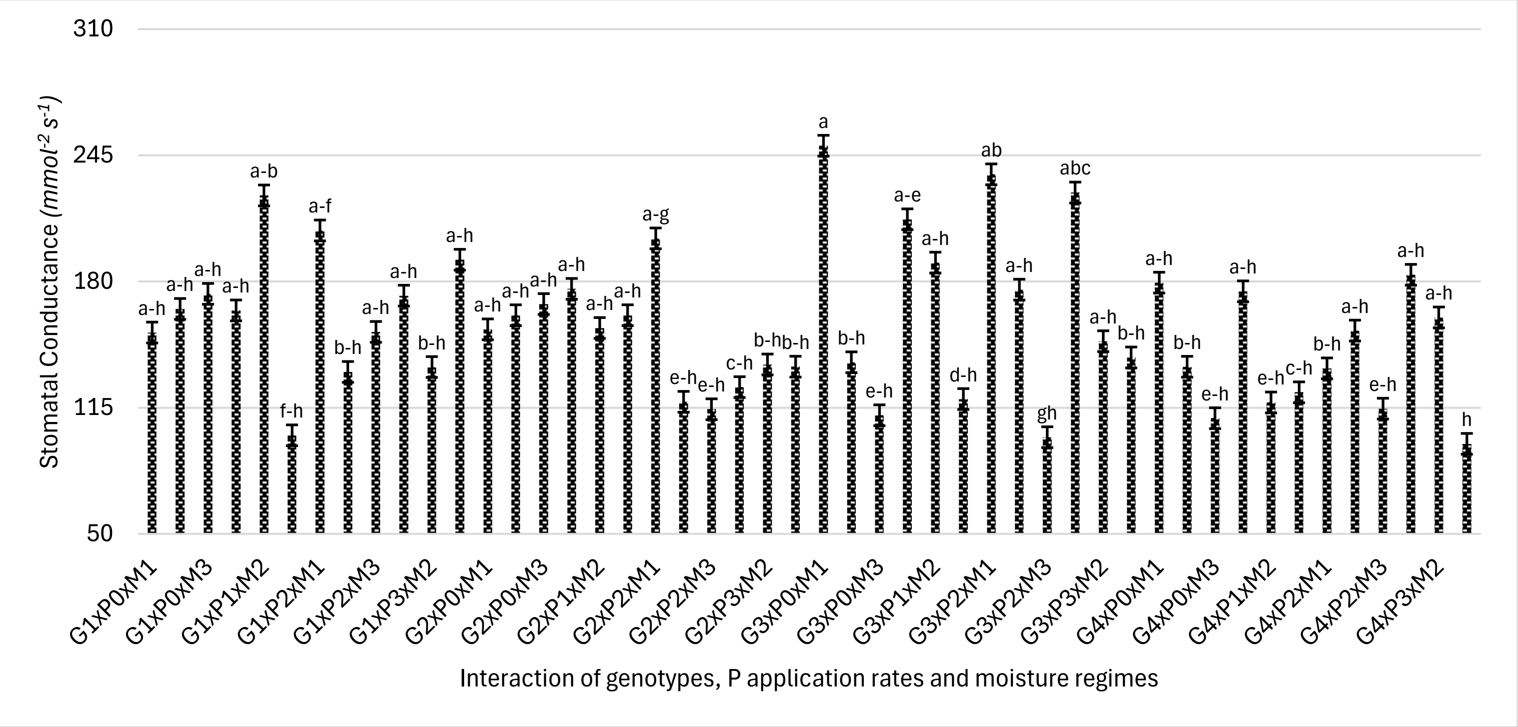


Figure 3.3: Treatment Interaction of genotypes, P application rates and moisture regimes on mean values of the stomatal conductance.

3.4.5 Regression, correlation, PCA, and cluster analysis

The response curve in Figures 3.4, 3.5, and 3.6 pins out the parameter responses to different levels of P fertilization. Growth parameters outlined in Figures 3.4 and 3.5 display a reaction curve of P rates fertilization on the number of leaves per plant, dropped leaves, number of branches, plant height, leaf length, leaf width, leaf area, and stem diameter. Moreover Figure 3.6 depicts the response curve of physiological attributes to different P fertilization rates. The R^2-values for the growth parameters ranged between 0.109 to 0.983. In contrast, the chlorophyll content and stomatal conductance R^2-values are 0.276 and 0.586 respectively (Figure 3.6).

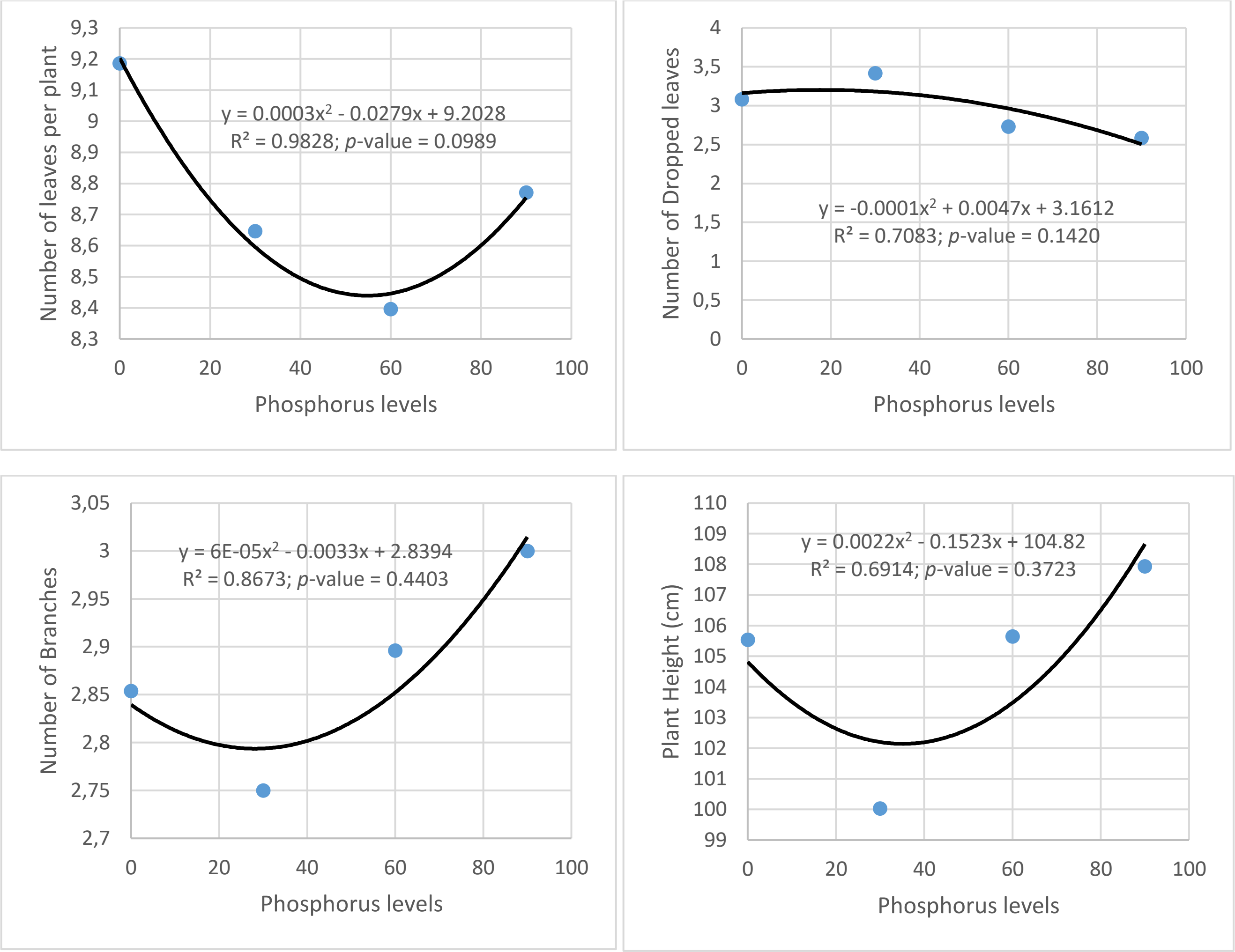
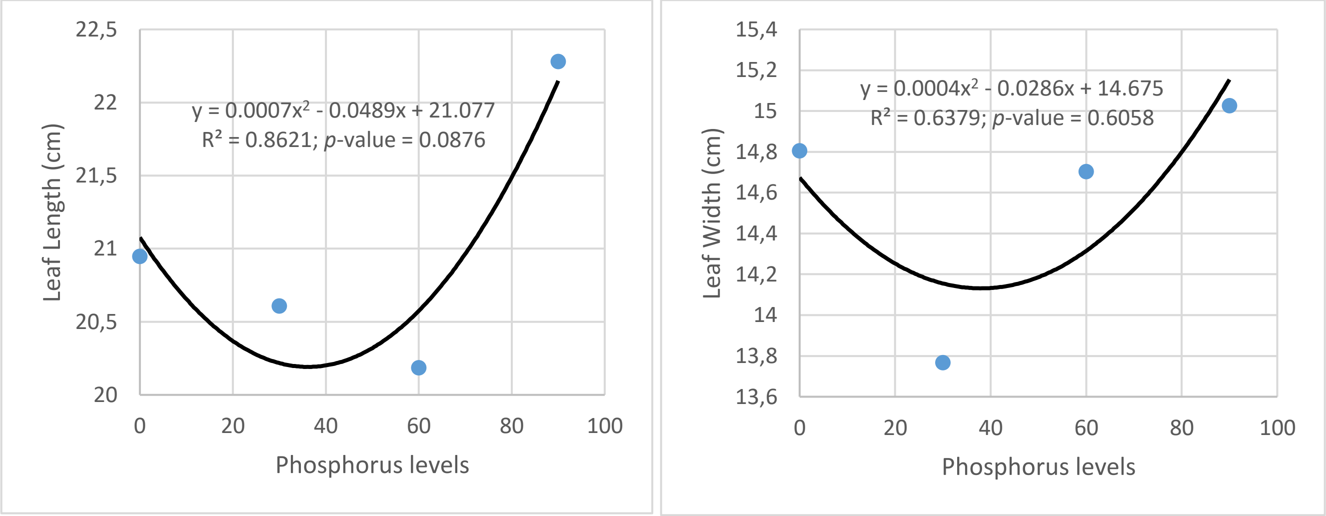


Figure 3.4: Quadratic polynomial of phosphorus (P) fertilizer application rate on growth attributes.



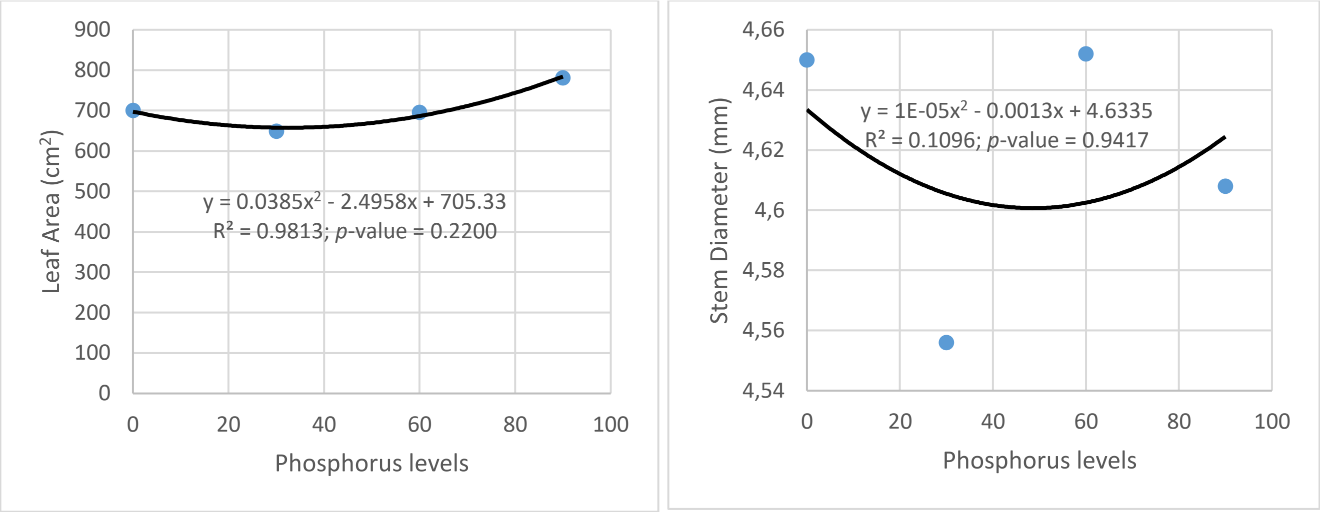


Figure 3.5:Quadratic polynomial of phosphorus fertilizer application rates on growth attributes.

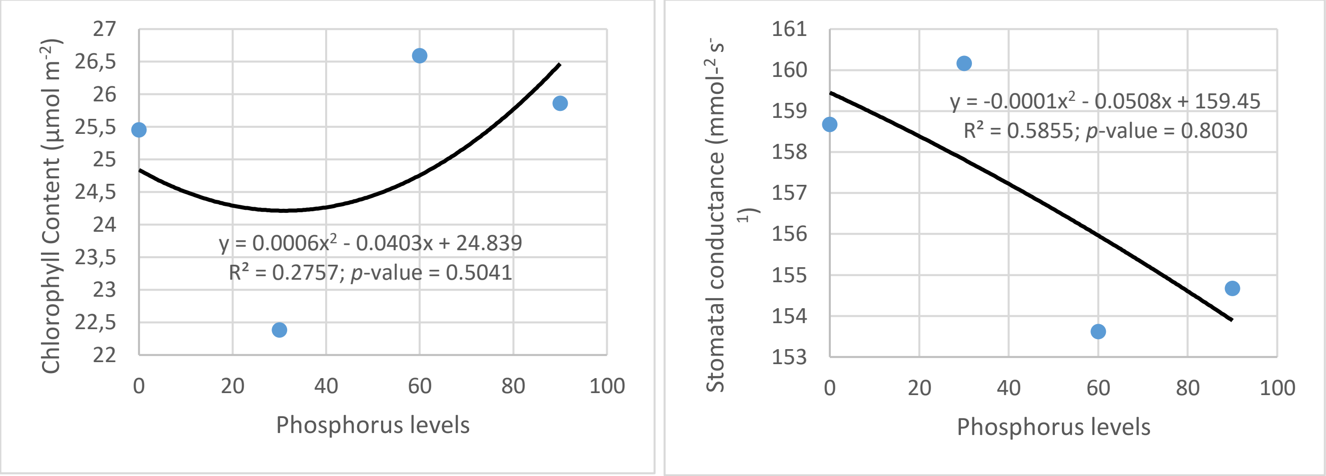


Figure 3.6:Quadratic polynomial of phosphorus fertilizer application rates on physiological attributes.

The results of the Pearson correlation matrix among various growth and physiological parameters are shown in table 3.7. Number of trifoliate leaves exhibited a positively significant (p≤0.05) correlation namely, the number of branches, plant height, and chlorophyll content (Table 3.7). Conversely, the number of dropped leaves showed a statistically significant and negative correlation with chlorophyll content. While negative and not statistically significant (p≥0.05) correlations were observed between plant height and leaf length, leaf length, leaf area, leaf area, and stomatal conductance (Table 3.7). A weak and non-significant (p≥0.05) correlation was detected between chlorophyll content and stomatal conductance (Table 3.7). The components loadings for the ten principals of the cowpea traits are shown in table 3.8. Among the ten principal components (PCs) the highest variability recorded is 32.595% with the most important traits' loads on PC1 being NofLvS (0.8411), NfBchs (0.6539), PntH (0.8142) and CCl (0.7234), respectively (Table 3.8). The PCA presented in Figure 3.7 underscores a strong correlation between leaf area (LA), Leaf width (LFW), stem diameter (StmD) and leaf length (StLgh) with close-aligned vectors. On the other hand, the CCI, number of trifoliate leaves (NoLfV) and plant height (PntH) indicate capturing the opposite aspect of the data underpinning their strong relationship.

The PCA biplot graphical display of the measured cowpea growth and physiological attributes is shown in Figure 3.7. Components C1 and C2 shown in the figure present a relatively high association within the components. Leaf area and LFW have stronger association with C2 whereas CCI is more associated with C1. Similarly, the results of the cluster analysis revealed that P fertilizer and Moisture regimes treatment combinations had limited association effects compared to the P fertilizer and genotypes hence revealing a preference for first response genotypic association order (Figure 3.8). The CV17B genotype irrigated at 15 kPa regime (G3M1) and CV17B genotype with 60 kg P ha^{-1} irrigated at 75 kPa regime (G3P2M3) showed strong and positive association (Figure 3.8). Interestingly, the association between 90 kg P ha^{-1} application rate irrigated at 15 kPa (P3M1) vis-à-vis CV17I genotype irrigated at 15 kPa moisture regime (G1M1) is stronger compared to CV17I genotype without P application under 50 kPa water regime (G1P0M2) versus CV17F with 90 kg P ha^{-1} at 75 kPa regime (G2P3M3), which has a comparably high association (Figure 3.8).

Table 3.7: Pairwise correlation matrix (r) among cowpea growth and physiological attributes

	Number of trifoliate leaves	Dropped leaves	Number of Branches	Plant Height	Leaf Length	Leaf Width	Leaf Area	Stem Diameter	Chlorophyll Content
No of trifoliate leaves	1								
No of dropped leaves	0.127	1							
No of Branches	0.626***	0.248***	1						
Plant Height	0.599***	-0.012	0.344***	1					
Leaf Length	-0.042	0.087	0.013	-0.090	1				
Leaf Width	0.080	0.034	0.015	-0.002	0.363***	1			
Leaf Area	0.002	0.046	-0.002	-0.072	0.743***	0.851***	1		
Stem Diameter	0.080	0.081	0.066	0.001	0.489***	0.348***	0.444***	1	
CCl	0.366***	-0.415***	0.222**	0.189**	0.079	0.063	0.070	0.185*	1
SC	-0.046	0.078	-0.028	-0.105	-0.074	0.006	-0.012	-0.033	0.005

= indicate significant effect at 5% level, **= indicate significant effect at 1% level and *= indicate significant effect at 0.1% level.*

Table 3.8: Loadings of the traits onto ten principal components among the cowpea traits

Traits	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**
NofLvS	0.841	0.435	0.152	-0.007	-0.027	-0.057	0.196	0.105	-0.162	-0.029
DPLVS	-0.064	-0.132	0.936	0.024	-0.191	0.012	-0.144	0.211	0.032	0.001
NfBchs	0.654	0.401	0.545	0.039	-0.059	0.082	-0.038	-0.316	0.025	0.006
PntH	0.814	0.459	-0.167	-0.079	-0.063	-0.148	0.161	0.116	0.163	0.020
LfLgth	-0.689	0.536	0.100	0.089	-0.132	0.372	0.245	0.029	-0.009	0.053
LfW	-0.305	0.834	-0.109	0.172	-0.086	-0.329	-0.219	0.009	-0.054	0.054
LA	-0.569	0.787	-0.062	0.153	-0.129	0.031	-0.029	-0.001	0.051	-0.093
StmD	-0.203	0.399	0.248	-0.249	0.821	0.009	-0.008	0.033	0.012	0.069
CCl	0.723	0.192	-0.326	0.284	0.108	0.399	-0.269	0.094	-0.003	0.005
StoM	-0.009	-0.264	0.129	0.907	0.245	-0.119	0.125	-0.005	0.018	0.000
Eigenvalue	3.259	2.446	1.434	1.035	0.832	0.453	0.284	0.179	0.060	0.016
Variability(%)	32.59	24.46	14.34	10.35	8.323	4.53	2.84	1.79	0.60	0.155

PC = principal components, NofLvS = Number of leaves per plant, DPLVS = Dropped leaves, NfBchs = Number of Branches, PntH = Plant Height, LfLgth = Leaf Length, LfW = Leaf Width, LA = Leaf Area, StmD = Stem Diameter, CCl = Chlorophyll Content and StoM = Stomatal conductance

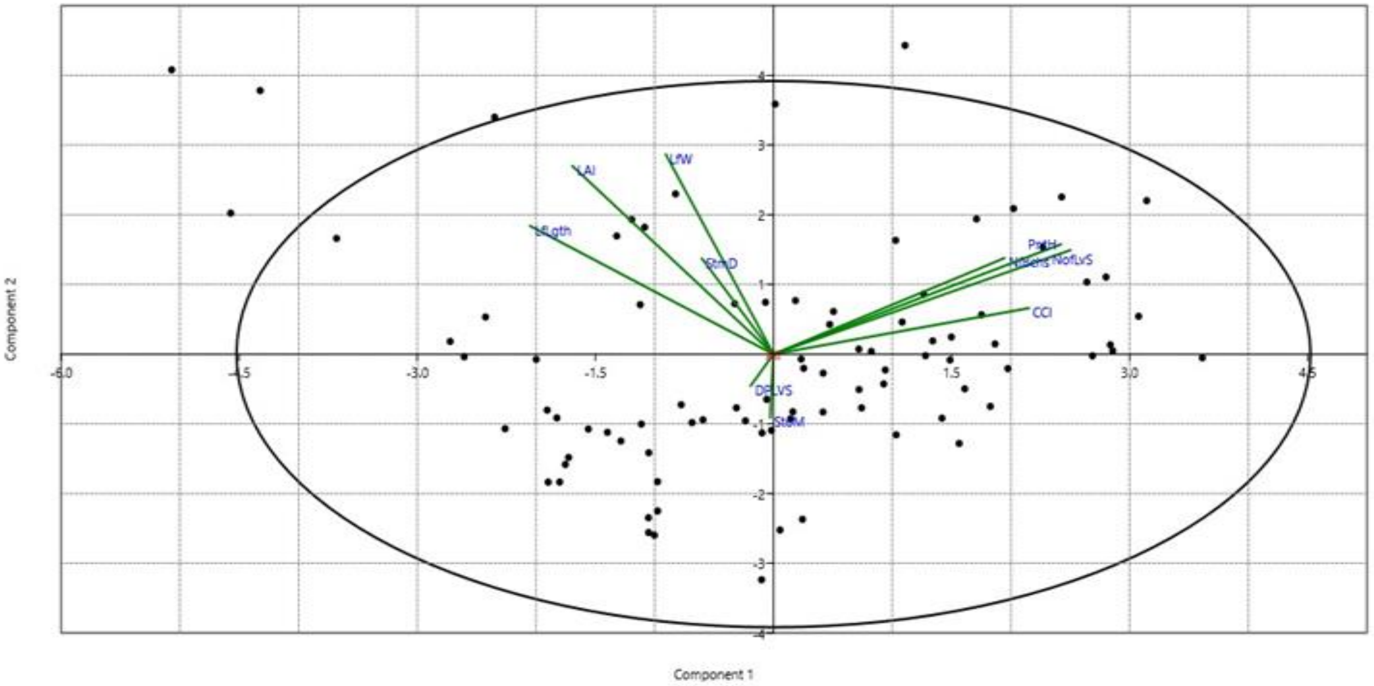


Figure 3.7: Biplot from PCA showing graphical display of the measured cowpea growth and physiological attributes.

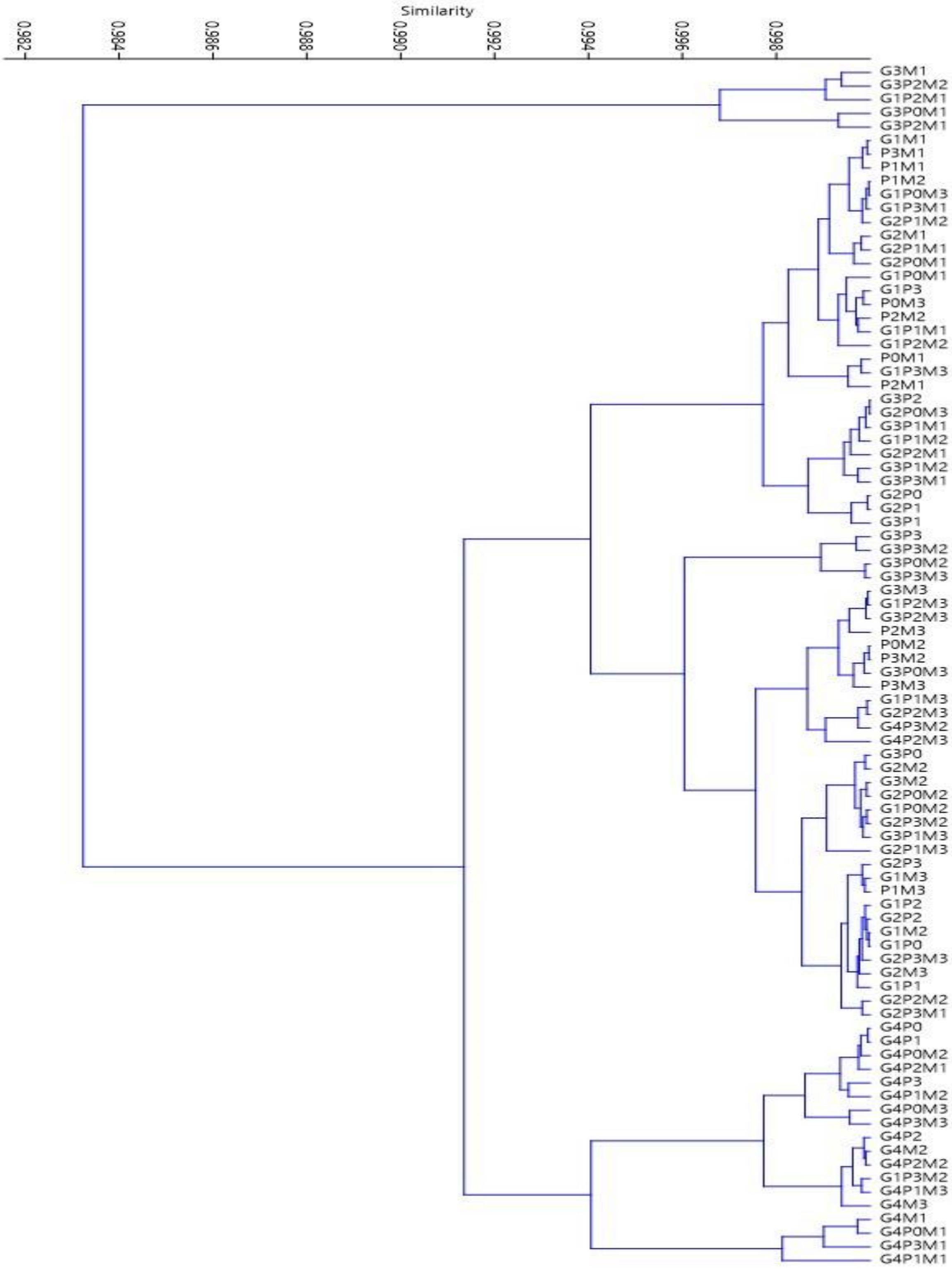


Figure 3.8: Cluster analysis of cowpea genotypes, P levels and moisture regime interaction effect using group averages and Euclidean distance methods.

3.5 Discussion

3.5.1 Results of pre-planting soil analysis

The results of laboratory analysis of the soil used for the study suggest it is slightly acidic but within the optimal pH range of 5.5 to 8.3 for cowpeas (Singh *et al.,* 2023). The measured available P level in the experimental soil is within the recommended rate of 15 to 18 mg kg^{-1} for soyabean production (FERTASA, 2016). Nevertheless, the exchangeable cations in the soil are high, generally revealing the adequacy of plant nutrient cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}). Finally, the results suggest the soil possesses a sandy loam texture; hence, it is well-drained with the potential to have good aeration that could be beneficial for promoting root and general plant growth and health.

3.5.2 Main treatments and their interaction on cowpea growth attributes

Trifoliate leaves measured per plant

Cowpea trifoliate leaves, also known as ternate leaves, are a leaf shape characterized by a leaf divided into three leaflets that vary in shape depending on plant species and genotype. These leaves play an important role in cowpea productivity. The observed significant GxM interaction effect on the mean number of trifoliate leaves suggests that genotype leaves response can be attributed to the level of moisture imposed. A similar observation was reported by Lakitan (2019) in common beans and Yahaya *et al.* (2019) in cowpea. The 10% reduction in the number of trifoliate leaves reported in the current study following soil moisture stress imposition agrees with earlier work by Yahaya *et al.* (2019). Hence, Bhattacharya (2021) argued that soil moisture plays a significant role in ensuring proper balance for optimum leaf production. Although the 90 kg P ha^{-1} rate in this study might be low compared to the 120 kg P ha^{-1} rate recommended by Khan *et al.* (2023) for high-P fixing African soils, such a higher rate exerted no significant increase in the mean number of trifoliate leaves in the current study. The significant effect of prolonged moisture stress on the mean number of leaves of cowpeas despite the reported drought tolerance (Carvalho *et al.,* 2017). The observation in the current study underscores that drought stress at 75 kPa reduces new leaf initiation to escape the detrimental effects of drought stress, thus a reduced number of trifoliate leaves. Conversely, the inconsequential GxPxM interaction effect observed on the number of trifoliate leaves in the current study might be due to limited nutrient availability under moisture stress conditions. However, the highest number of trifoliate leaves observed with CV18-1A genotype without P application under moderate moisture stress suggests a higher drought tolerant attribute of this genotype than any other, which agrees with the finding by Oloyede-Kamiyo *et al.* (2021), who reported that the genetic makeup of cowpea influence leaf production. Furthermore, Havlin (2020) reported that plants use what is available in the soil for their productivity; hence, the CV18-1A genotype may have relied on the native soil P for increased leaf production under moderate moisture stress.

Number of leaves dropped per plant

Typically leaf dropping in plants is an indication of a plant's health response to either inadequate moisture or nutrient deficiencies (Adnan *et al.,* 2020; Corso *et al.,* 2020; Battaglia *et al.,* 2019). Thomas and Donnison (2021) also hinted that plants' leaves turn yellow before dropping off due to maturity inducement by plants to redirect energy toward reproduction. Furthermore, Mayta *et al.* (2019) outlined that leaf cells endure several potential pathways toward its demise during senescence. Interestingly, current findings revealed that variation in cowpea genotypes was the only factor that significantly influenced the number of leaves dropped, with only the CV18-1A genotype demonstrating greater leaf-holding ability by withstanding greater water- and P-limiting conditions. Additionally, the different cowpea genotypes have different maturity times, which may have contributed to the significantly lowest number of dropped leaves recorded in CV18-1A genotype. Notwithstanding the observed inconsequential GxMxP interaction effect on the number of leaves dropped, the CV18-1A genotype without any P application under moderated moisture stress exhibited the greatest ability to hold leaves. The result aligns with the findings by Seleiman *et al.* (2021) who attributed the loss of plant leaves arising from moisture stress to the disruption of physiological, metabolic, and biochemical processes critical to plant productivity. Additionally, Thomason and Battaglia (2020) reported that the change in leaf colour and the subsequent dropping of leaves affects the amount of protein stored including reduced indigestible structural polysaccharides, physiologically active substances, and antioxidants increase.

Number of branches produced per plant

The observed significant effect of the variation in moisture regimes and cowpea genotypes on the number of cowpea branches aligns with existing literature (Mofokeng *et al.,* 2020; Gerrano *et al.,* 2019). Soil moisture availability is a crucial factor influencing branch development; hence, moderate to severe moisture stress could limit branch growth, leading up to a 12% reduction. The non-significant effect of variation in P levels on the mean number of branching could be due to the possible adequacy of the inherent P supply in the soil used for the trial, which agrees with an earlier report by Hashemabadi (2013). However, the finding contradicts earlier results by Tekulu *et al.* (2020) and Namakka *et al.* (2017), who reported a significant P effect on the growth and branching ability of cowpeas and groundnuts. The inadequate significant variation of GxP on the number of branches per plant converses with the findings of Rabbani *et al.* (2023), reporting a significant interaction effect imposed by the P application rate on eight soybean varieties. However, Aduloju *et al.* (2009) found similar findings to the present study, with no statistical variation between GxP interaction on the number of branches. Thus, these findings suggest that the lack of significant responses may be due to P occlusion, generally being fixed into forms unavailable for the tested genotypes. The non-significant GxPxM interactions effect on cowpea branching suggests no to limited complex interdependency of these factors. Nonetheless, CV17F genotype at 90 kg P ha^{-1} fertilizer application under moderate moisture stress had fewer branches possibly compared to CV17I fertilized with 30 kg P ha^{-1} under severe moisture stress that had more branches possibly due to nutrient imbalance in the former.

Plant height

Plant height is an important phenotypic and morphological parameter that directly indicates the plant’s overall growth, which can have a greater implication on the final plant's grain yield and biomass production (Wang *et al.*, 2018). The observed significant genotype as well as phosphorus and soil moisture regime interaction effect on the measured plant height are comparable to earlier study by Jadhav *et al.* (2023) who evaluated 10 cowpea genotypes with variable genetic and morphological makeup. The CV18-1A with unique growing traits (e.g., stature and architecture) displayed a remarkably tallest plant height. Similar observation was reported by Alqudah *et al.* (2016) for barley. The inconsequential P levels and moisture regimes individually as well as any of the GxP, GxM, and GxPxM interaction effect on agree with previous study by Augustine and Godfre (2019). The 30 kg P ha^{-1} fertilization under severe moisture stress (75 kPa) severely reduced the plant height by 26% compared to 30 kg P ha^{-1} fertilization irrigated under adequate moisture regime (15 kPa). Bana *et al.* (2018) similarly reported a 13% reduction in plant height in wheat (Triticum aestivum) due to severely limited water availability. The observed GxPxM interaction effect on plant height underscores the intricacies in these factors, suggesting that though genotypes exhibit inherent growth advantages over others, the modulation of this trait by P availability and moisture stress can be variable and nuanced.

Leaf length and width

Leaf length and width are important morphological traits that play a critical role in plant growth and development including adaptation and response to various growing conditions. The significant GxP interaction effect on leaf length recorded in the current study with the longest leaf length obtained with CV17B genotype at 90 kg P ha^{-1} suggests that CV17B genotype leaf length responds positively to higher P application levels and exhibits superior ability to utilize more P to enhance leaf length and width. Similar finding was reported by Duan *et al.* (2022) on Fengdan (*Paeonia ostii T. Hong and J. X. Zhang*), a perennial oil and medicinal plant. Lee *et al.* (2017) further highlighted the interaction between soil moisture and nutrient availability such as N and P attributing a significant role in leaf width and length. These findings underscore that moisture stress can alter nutrient uptake and utilization efficiency, thereby influencing plant growth and development, including leaf characteristics. The significant GxM interaction effect on leaf length produced a reduced leaf length in CV17B genotype under severe moisture stress regime with P application suggesting that moisture stress modified the response to P availability. Nguyen *et al.* (2022) reported that low P application resulted in significantly smaller leaf length in wheat breeding lines.

Regrettably, none of the main treatment and their interaction effect exerted any significant effect on the measured leaf width in the current study. This observation contradicts previous studies reported by Duan *et al.* (2022) and Nguyen *et al.* (2022) notwithstanding the unexpected observations. For instance, CV17I genotype under moderate soil moisture stress and 60 kg P ha^{-1} fertilizer application had narrow leaf width while under severe moisture stress with the same P level, it showed greater width. This suggests that genotype plays a role in determining leaf width while factors such as soil moisture and P conditions modulate the responses, which further underscores the complex nature of GxPxM interaction effect in shaping plant morphology. Digrado *et al.* (2022) and Gerrano *et al.* (2019) reported a considerable genetic variation in the agronomic traits of cowpea genotypes including leaf length and leaf width. The contrasting insignificant leaf length observed under different moisture stress levels and P applications are consistent with that emphasized insignificant differences imposed by genotype x moisture regime x P fertilization rate interactive effect reported in the present study.

Cowpea leaf area

The LA is one of the key indicators for plant growth, which varies greatly among different genotypes due to genetic differences in leaf size and canopy architecture. It is influenced by differential conditions such as light availability, temperature, humidity, soil moisture, and nutrient availability such as soil P. Results from the current study revealed that only the moisture regime and the GxP as well as PxM interaction effect significantly affected LA. In particular, CV17B with 60 kg P ha^{-1} application had the smallest LA, whereas CV17B with 90 kg P ha^{-1} gave the highest LA, underscoring the positive effect of P availability on LA. Moreover, LA was significantly reduced under severe soil moisture stress, emphasizing LA's sensitivity to water availability. However, the inadequate effect of variable P fertilization levels on LA contradicts the findings of Zhang *et al.* (2018) reporting P application significantly improved the LA remarkably. Similarly, Teli *et al.* (2020) reported that P availability enhances leaf expansion and overall growth. However, the present findings suggest that P applied in this study was inadequate to relatively low to improve the LA. This could ultimately be due to the fixed P in response to the slightly acidic in the soil used. The reduction in LA under moisture stress conditions observed in this study aligns with existing knowledge on plant responses to reduced water availability to maintain water balance (Li *et al.* 2022; Sousa *et al.* 2022).

Stem diameter

Stem diameter variations (SDV) are widely recognized as a useful drought stress indicator and have therefore been used in many irrigation scheduling studies (De Swaef *et al.*, 2015). It is influenced by genetic makeup and plant species that dictate plant architecture and growth habits hence, different genotypes within a species can exhibit variations in stem diameter. The current study revealed a significant P level and moisture regime interaction effect on stem diameter. Specifically, the application of 60 kg P ha^{-1} under severe moisture stress resulted in the widest diameter which might mean that when stress is imposed on cowpea it increases stem diameter to allow more water uptake, contrary to 60 kg P ha^{-1} under moderate moisture stress had the thinnest diameter, suggesting that the combined effects of P availability and moisture stress influence the structural development of cowpea stems. Phosphorus availability can enhance cell division and expansion, contributing to thicker stems, especially under stress regime where nutrient uptake efficiency becomes critical. The non-significant P fertilizer effect on stem diameter obtained in the present study contradicts previous findings (Tariq *et al.*, 2023; Liu 2021) where P was reported to enhance stem diameter significantly. This may be related to the adequate P level in the soil used for the experiment based on the critical soil P level of 15 to 18 mg kg^{-1} for soybean reported by FERTASA (2016) hence, the addition of P produced no positive response. The unexpected widest diameter observed under severe moisture regime (though not significantly different from other moisture treatments) contradicts earlier works (Baba *et al.,* 2024; Seleiman *et al.,* 2021) where moisture stress was reported to constrain cell expansion and elongation leading in thinner stems. However, the result agrees with the finding by Li *et al.* (2020) who reported that mild or moderate irrigation deficit increases cotton stem diameter during flowering stage.

3.5.2 Main treatment and interaction on cowpea physiological parameters

Chlorophyll content

Chlorophyll is an essential plant molecule that is responsible for collecting solar energy in photosynthetic antenna systems for charge separation and electron transport within reaction midpoints. It plays a significant role in photosynthesis by capturing light energy for plant growth and development. Soil moisture availability has a significant effect on chlorophyll levels, according to Ashkavand *et al.* (2015) and Mndela *et al*. (2023), thereby affecting photosynthetic efficiency and general plant productivity due to the increased ability of plants to manufacture their own food. This study revealed that genotype and moisture regime were the primary factors that influence ($p \leq 0.05$) chlorophyll content. CV18-1A genotype exhibits a significantly higher (33.36%) chlorophyll content compared to CV17B under optimal moisture regime. Variations in chlorophyll content among different plant genotypes were influenced by genetic factors thus affecting biosynthesis and degradation rates, as well as leaf physiology. Moreover, the findings further revealed that severe moisture stress led to about 43% reduced cowpea chlorophyll content compared to the moderate and well-watered regimes indicating the sensitivity of chlorophyll levels to moisture availability. Several earlier studies have proved that moisture stress degrades chlorophyll content (Hu *et al.,* 2023; Wu *et al.,* 2016). Furthermore, literature evidence for maize (Xiong *et al.,* 2023) and wheat (Wen *et al.,* 2024) underscore significant genotypic differences in chlorophyll content under diverse moisture and soil nutritional conditions. Interestingly, P availability in the current study did not significantly influence chlorophyll content suggesting that while moisture availability and cowpea genotype interplay a significant role in chlorophyll synthesis, P levels may not influence chlorophyll biosynthesis of cowpea due to the lack of adequate P in situ. This can be justifiable by a review of Veneklaas *et al.* (2012) reporting that plants take up only about 30% of applied P, while 60% is adsorbed to the soil, which means the significant effect of P might not be instantly feasible on the chlorophyll content.

Stomatal conductance

Stomatal conductance is a significant physiological process of plants regulating gaseous exchange, through CO_2 uptake for photosynthesis and the loss of water vapour through stomatal pores. The dynamics of stomatal conductance are influenced by different genetic factors, moisture availability, and P levels (Asargew *et al.,* 2024; Bertolino *et al.*, 2019). The significant ($p \leq 0.05$) genotype, moisture regime, and their interaction effect as well as GxPxM interaction on stomatal conductance observed in the current study suggests P has a greater effect when integrated with genotype and moisture regimes solely. For instance, CV18-1A genotype fertilized at 90 kg P ha^{-1} under severe moisture stress (75 kPa) exhibited 48% reduced stomatal conductance relative to CV18-1A genotype at 90 kg P ha^{-1} under well-watered regime (15 kPa), which suggests that stomatal closure responds to moisture stress regime to possibly conserve water under limited moisture availability significantly. Additionally, CV17B genotype without P application under well-watered regime (15 kPa) gave the highest stomatal conductance indicating optimal gas exchange under a favourable moisture regime, albeit potentially leading to higher water loss. These findings underscore genotype-specific responses to moisture stress and highlight different genotypes' diverse strategies to regulate stomatal conductance under varying moisture and soil nutrient conditions. Additionally, the abscisic acid hormonal synthesis in response to moisture stress leads to stomatal closure to conserve water, thereby reducing stomatal conductance and limiting the CO_2 uptake, which lowers photosynthesis which might be the case in the current findings (Pirasteh-Anosheh *et al.,* 2016).

The inconsequential effect of neither P application nor its interactions with both genotype and moisture regime variation might be related to P diffusion in the soil to overcome the restricted P difference created due to P uptake in the soil leading to unavailable P; otherwise, the ultimate effect without destruction to the hormonal response. Thus, P deficiencies can increase abscisic acid (ABA) concentration. This hormone promotes stomatal closure further reducing stomatal conductance, water loss under stress regime, and overall crop productivity (Pirasteh-Anosheh *et al.,* 2016). Contrarily, earlier reports by Khan *et al.* (2023) and Shu *et al.* (2023) suggest that P deficiency can lead to decreased stomatal density, size, and aperture, ultimately resulting in reduced CO_2 uptake rates and diminished photosynthetic efficiency. Similarly, Kaur *et al.* (2021) reported that plants close stomata under water-deficit regime to prevent major water loss, which consequently leads to a reduction in photosynthesis through decreased influx of CO_2. Moreover, considering the role of P in the overall plant growth and function, its direct effect on stomatal conductance may be more nuance and context dependent. For instance, earlier studies on wheat, modulated maize, and rice have revealed how different genotypes exhibit varying responses in stomatal conductance including through reduced transpiration under drought conditions (Liao *et al.,* 2024; Ma *et al.,* 2018; Ouyang *et al.,* 2017). Consequently, plants compensate for the water deficit regime with the closure of stomata as an avoidance strategy for water loss through transpiration. This might be the case in our findings, where severe moisture stress (75 kPa) significantly reduced the stomatal conductance. This adaptation is crucial for plants to cope with limited water availability and maintain water balance. Additionally, Buckley (2019) reported that increasing transpiration reduces leaf water potential and stomata partially close, making the net change in water potential smaller than otherwise.

3.5.3 Pearson correlation matrix among all measured phenological and yield parameters

The outlined results of the Pearson correlation matrix show that the number of dropped leaves attributing to a statistically significant negative correlation with chlorophyll content indicates that leaf senescence reduces the photosynthetic processing of cowpea, as reported by Mayta *et al.* (2019) in a study of chloroplast relation to age and growth of plants. The negative correlation could also reflect environmental stressors or conditions at which the plant is grown, where chlorophyll is reduced, resulting in leaf shedding.

A negative but inadequate correlation effect was observed between plant height and leaf length, highlighting that the taller plant does not necessarily reflect longer leaf length. This has been observed in a study Wang *et al.* (2024), suggesting that growth attributes, including plant height and leaf length, might not have a strong correlation due to varying growing conditions or the genetic makeup of the tested crop. However, Coka (2024) reported contradicting findings that there was a strong positive correlation amongst almost all the assessed growth parameters, including the plant height and leaf length. Likewise, an inadequate significant correlation between leaf area, stomatal conductance, and leaf length further emphasizes the density of physiological and growth plant traits functioning independently.

The weak and non-significant correlation observed between chlorophyll content and stomatal conductance may suggest that the regulation of gas exchange does not always directly impact chlorophyll levels. Previous studies have shown stomatal conductance influences photosynthetic efficiency which impacts chlorophyll content despite the indirect influential external factors such as light intensity and moisture availability (Tang *et al.,* 2023; Matsumoto *et al.,* 2005).

3.6 Conclusion

A significant variation in growth and physiological response of the different cowpea genotypes was observed in the current study, indicating that cowpea genotypes have diverse genetic makeups directly affected by soil moisture and nutrient uptake for active cowpea growth. Moreover, our findings reveal that one special mechanism cowpeas use during the water deficit regime is reducing stomatal opening to decrease stomatal conductance. However, some growth and physiological attributes were not statistically significantly affected by the treatment interaction effect. Therefore, the alternative hypothesis that treatment applications would similarly affect the different genotypes tested is rejected. With concrete findings of this study revealed that the differential cowpea genotypes resulted in differential outputs effect of treatment applications. Thus, CV17B and CV18-1A genotypes are considered the best-performing genotypes among the tested genotypes on growth and physiological responses. This study concludes that cowpea growth and physiological response can be highly dependent on sole genotypic and water regime factors, however, the 60 kg P ha^{-1} rate reveals a high response when interacted. Despite that, most measured parameters did not differ statistically amongst others, especially the treatment combination.

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CHAPTER 4

Variable phosphorus fertilizer levels and soil moisture regimes affect phenological, yield attributes and water-use efficiency of four cowpea (*Vigna unguiculata* L.) genotypes grown under greenhouse conditions

Abstract

The effects of severe drought and soil fertility constraints on crop production are known to exert a negative effect on crop production and food security. The study examined the effect of variable phosphorus (P) fertilizer levels and soil moisture regimes on cowpea phenological, yield attributes and water-use efficiency (WUE). Trial consisted of four each of cowpea genotypes, G (CV17I, CV17F, CV17B and CV18-1A) and P levels, F (0, 30, 60, and 90 kg ha^{-1}) as well as three soil moisture regimes as treatment factors. The soil moisture regimes (M) comprised of well-watered at 15 kPa, moderate stress at 50 kPa, and severe moisture stress at 75 kPa; and imposed at flowering stage for 21 days. Results revealed that the first-order PxM interaction exerted a significant ($p \leq 0.05$) effect on the mean pod length and number of seeds per pod, while GxM interaction only influenced the mean number of pods per plant. The CV17I genotype under severe moisture stress experienced 37.5% seed yield reduction per plant relative to the well-watered treatment but hundred seed weight (HSW) surprisingly recorded 5% increase under moderate moisture stress. The CV17F and CV17B genotypes had comparable HSW with about 39% higher compared to the other tested genotypes. The variation in genotypes, soil moisture regimes and GxP interaction exerted significant ($p \leq 0.05$) effect on cowpea total aboveground biomass yield and water-use efficiency at harvest while severe moisture stress resulted in delayed flowering and pod formation, reduced water use efficiency and reduced number of seeds per pod.

Keywords: Cowpea productivity, water stress, grain yield, water use

4.1 Introduction

Cowpea is a legume crop that plays a significant role in the cropping systems of water-limited tropical regions including providing significant plant protein source to complement the cereal-based foods in many homes for animals and millions of people in poor rural communities (Mekonnen *et al.,* 2022). Nkomo *et al.* (2021) reported that 7.4 million tons of cowpeas are produced globally with Africa being the leading producer of nearly 5.2 million tons. However, most cowpea growers experience extremely low on-farm grain yield that rarely exceeds 527 kg ha^{-1}, which is far less than the potential achievable yield of more than 2500 kg ha^{-1} (Ndor and Faringoro, 2020). Such poor grain yield is attributed to numerous factors, such as inadequate farmers' practices and poor inherent soil conditions that include limited phosphorus (P) supply and inadequate soil moisture, despite the widely reported drought-tolerant ability of cowpeas.

The current reality of food and nutrition insecurity in many African countries including South Africa is of great concern and calls for urgent intervention. South Africa as a developing country still experiences high poverty level with two-thirds (64.2%) of the over 30 million people reportedly living below the poverty line being African black population (Mbajiorgu and Odeku, 2023). Stats SA (2021) similarly reported that about 2.6 million people in South Africa have inadequate access to food, with an additional 1.1 million being severely affected. This pathetic and highly uncertain situation demands an urgent response to advance integrated sustainable practices underpinned by robust and data-driven research that promotes sufficient food production over the current 0.73% global population growth rate (Dada *et al.,* 2021). Moreover, cowpea growers who are predominantly smallholders often rely on native soil P with little to suboptimal mineral fertilization resulting in low yields (Kutu, 2012). Cabeza *et al.* (2024) reported that inadequate supply of P can result in poor N_2 fixation legumes particularly in P-deficient soils. The situation is exacerbated by weak solubility to no P availability from mineral phosphates (Johan *et al.,* 2021). Phosphorus in cowpea production is not only essential in root formation, plant growth, and improved biomass and grain yield Aryal *et al.* (2021); Mohammed *et al.* (2021), but also essential for nodule formation and enhanced efficiency of rhizobium-legume symbiosis (Karikari *et al.*, 2015; Augustine and Godfre, 2019). However, Poudel *et al.* (2024) reported that cowpea production requires a minimum of 40 to 60 kg P ha^{-1} for maximum productivity. Nevertheless, the challenge of attaining increased cowpea yields is made more complex by drought and heat stress arising from climate change. The situation is made worse by the chemical forms of P in soils and its complex chemistry in most African tropical soils that are highly weathered and prone to the phenomenon of P fixation (Johan *et al.,* 2021).

Terminal and intermittent droughts cause substantial yield reduction in crops due to the negative impact on overall plant growth, physiology and reproduction. Drought stress reduces the crop yield through a reduction in seed weight and biomass accumulation Du *et al.* (2024) while plant shoot and root growth are also limited by terminal and intermittent drought (Fang *et al.,* 2024). However, the effect of drought in any location varies with the duration and intensity of soil moisture deficit resulting from irregular or shortage of normal rainfall or late rains and plantings that are affected by the prolonged drought (Ntali *et al.,* 2023; Seleiman, *et al.*, 2021; Bhaga *et al.*, 2020). Notwithstanding drought-tolerant ability of cowpea, it can suffer severe yield losses from intermittent and terminal drought effects depending on the severity and the stage of growth (Nunes *et al.,* 2022). Thus, the study assessed the response of phenological, yield attributes, and water use efficiency of four various cowpea genotypes to soil moisture effect and P fertilization at two crop stages.

4.2 Methodology

4.2.1 Description of the method

The details of experimental description, treatments, trial layout and layout for the study are as previously provided in sections 3.2.1 to 3.2.4 in chapter 3.

4.2.2 Data collection

4.2.2.1 Phenological and related yield data

The phenological data observed and collected at the reproductive stage and yield data were collected at harvesting, respectively. The phenological data collected include the number of days to flowering and number of days to pod formation, and both were calculated from the initial emergence date. The related yield data obtained at harvest include the number of pods per plant, number of seeds per pod, seed weight per plant, hundred seeds weight (HSW) per plant, and the number of cavities were counted for each pod per plant. The pod length (cm) was measured using a steel ruler while the seed weight per plant and the HSW were measured using a Kern analytical weighing balance (model 220-4M).

4.2.2.2 Biomass collection

The biomass yield data was collected during the reproductive growth stage of the plants as described in Chapter 3. One plant per pot was cut from the base to the brown bag. The cut plants were then transported to the research laboratory for oven drying. The samples were oven-dried for 48 hours at a temperature of 65°C. Afterward, they were weighed (g) using a digital weighing balance (model 220-4M).

4.2.2.3 Quantification of total biological yield at harvest and water-use efficiency

The total biological yield at harvest (TBYh) measured as gram per plant was computed using a formula outlined by Bijalwan and Dobriyal (2014):

$$TBYh\ (g) = GY + PS\ (haulm + chaff) \qquad \textit{(Equation 4.1)}$$

Where: GY= grain yield (g/plant), PS = plant straw (g/plant) comprising pod haulm and chaff

Water-use efficiency (WUE) at flowering and harvest stages was computed using the following formular as described by Aldesuquy *et al.* (2013):

At flowering:

$$WUEfl\ (g\ mm^{1}) = \frac{BMfl\ (g)}{TAW\ (mm)} \qquad \textit{(Equation 4.2)}$$

Where: WUEfl = Water Use Efficiency at flowing, BMfl= Biomass yield at flowering (determined in 4.2.2.2 above) and TAW =Total amount of water applied

At harvest:

$$WUEh\ (g\ mm^{1}) = \frac{TBY\ (g)}{TAW\ (mm)} \qquad \textit{(Equation 4.3)}$$

Where: WUEh= Water Use Efficiency at harvest, TBYh = Total biological yield at harvest (calculated as per equation 4.1) and TAW = Total amount of water applied

4.3 Statistical analysis

A detailed description of the statistical analyses for all measured parameters is outlined in Chapter 3, Section 3.3.

4.4 Results

4.4.1 Results of *p*-values for the measured parameters

Table 4.1 below presents the *p*-values of the measured phenological and yield parameters from the trail. The results reveal that various tested genotypes had a significant ($p \leq 0.05$) effect in all the measured parameters while the variation in moisture regimes similarly exerted a significant ($p \leq 0.05$) effect in all the parameters except for the HSW. The various P fertilizer rates only exerted a significant ($p \leq 0.05$) effect on the biomass yield at flowering as well as the measured WUE only at flowering. Interestingly, a significant PxM interaction effect on the mean number of pods per plant, pod length and number of seeds per pod were observed. The GxM interaction exerted a significant ($p \leq 0.05$) effect on the mean number of pods per plant while a significant GxP interaction effect significantly affected the TBYh and WUEh.

4.4.2 Main treatment effects on measured phenotypic and yield attributes of grain cowpea

Table 4.2 depicts the mean values of the measured phenological and yield attributed as response to the main treatment effects. The results reveal marked variation in phenological characteristics of the four genotypes with CV17I exhibiting early flowering (49 days) and pod formation (52 days) while CV18-1A had the longest flowering and pod formation representing nearly 63 and 67 days, respectively. The CV17F genotype produced the highest seed weight per plant while the observed HSW from CV17F and CV17B genotypes is statistically same. Interestingly CV18-1A had the longest pod length and the mean number of cavities and seeds per pod. Equally, cowpea plants that received P fertilization at 60 kg ha^{-1} produced the highest number of pods and pod length per plant although there was no significant difference among the P rates. Furthermore, adequate soil moisture application at 15 kPa significantly increased the mean number of pods and pod length per plant.

Despite the inconsequential effect of P variation on cowpea in the current study, the results show that the application of 60 kg P ha^{-1} gave the highest number of cavities and seed weight per pod while the HSW obtained is marginally (<1%) lower than the value obtained with 90 kg P ha^{-1}. Interestingly, the 30 kg P ha^{-1} gave the highest number of seeds per plant. However, the effect of P fertilizer application on all measured variables was least (13.4%) on the mean number of pod length. The yield-related parameters obtained were highest under full (irrigation) soil moisture regime (15 kPa) with significant biomass (BMfl and TBYh) and seed yield reduction as the severity of moisture stress increases. While 37.5% cowpea seed yield reduction was recorded due to severe moisture stress, HSW surprisingly recorded about 1 and 5% increase under severe and moderate moisture stress, respectively. Interestingly, the results revealed a delay in the mean number of days to flowering and pod initiation depending on the severity of moisture stress with the longest days recorded at 75 kPa (Table 4.2).

Table 4.1: *p*-values for phenological and yield attributes of cowpea to genotypes, P rates and moisture regimes

S of V	NoDfl	NoDpd	NoPdpp	PdL	NoSdpp	NoCpp	SWtpp	HSW	BMfl	TBYh	WUEfl	WUEh
G	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.002**	0.000***	0.004**	0.000***
P	0.154^{ns}	0.091^{ns}	0.649^{ns}	0.856^{ns}	0.619^{ns}	0.728^{ns}	0.624^{ns}	0.354^{ns}	0.000***	0.952^{ns}	0.001**	0.948^{ns}
M	0.001**	0.008**	0.000***	0.001**	0.000***	0.000***	0.000***	0.231^{ns}	0.000***	0.000***	0.084^{ns}	0.000***
GxP	0.952^{ns}	0.979^{ns}	0.613^{ns}	0.060^{ns}	0.078^{ns}	0.811^{ns}	0.885^{ns}	0.961^{ns}	0.525^{ns}	0.033*	0.503^{ns}	0.038*
GxM	0.260^{ns}	0.390^{ns}	0.026*	0.649^{ns}	0.621^{ns}	0.805^{ns}	0.091^{ns}	0.242^{ns}	0.259^{ns}	0.437^{ns}	0.420^{ns}	0.451^{ns}
PxM	0.886^{ns}	0.903^{ns}	0.536^{ns}	0.041*	0.013**	0.173^{ns}	0.816^{ns}	0.923^{ns}	0.813^{ns}	0.249^{ns}	0.879^{ns}	0.282^{ns}
GxPxM	0.588^{ns}	0.591^{ns}	0.361^{ns}	0.454^{ns}	0.178^{ns}	0.689^{ns}	0.823^{ns}	0.273^{ns}	0.153^{ns}	0.642^{ns}	0.172^{ns}	0.630^{ns}

*S of V implies sources of variation; G= Genotypes, P = Phosphorus levels, M = Moisture regimes, ns = not significant, ns = not significant; *, ** and *** indicate significant effect of treatment at 5%, 1% and 0.1% level, respectively; NoDfl= Number of days to flowering, NoDpd= Number of days to pod formation, NoPdpp = Number of pods per plant, PdL = Pod length (cm), NoSdpp = Number of seeds per pod, NoCpp = Number of cavities per pod, SWtpp = Seed weight per plant(g), HSW= Hundred Seed Weight (g), BMfl= Biomass at flowering (g), TBY=Total biological yield at maturity (mg/g), WUEfl = Water use efficiency at flowering (g* mm^{-1}*), and WUEh = Water use efficiency at harvest (g* mm^{-1}*).*

Table 4.2: Effect of genotype, P levels, and moisture regimes on phenological and yield attributes

Treatment factors	NoDfl	NoDpd	NoPdpp	PdL	NoSdpp	NoCpp	SWtpp	HSW	BMfl	TBYh	WUEfl	WUEh
Genotypes												
CV17I	49.1^{b}	52.3^{b}	5.08^{a}	10.98^{b}	7.46^{b}	10.09^{b}	3.47^{b}	13.51^{b}	6.05^{a}	8.47^{a}	1.14^{ab}	0.91^{a}
CV17F	52.8^{b}	55.9^{b}	3.25^{b}	13.69^{a}	8.21^{b}	10.02^{b}	4.08^{a}	22.16^{a}	5.46^{b}	8.17^{a}	1.04^{b}	0.87^{a}
CV17B	50.5^{b}	53.4^{b}	2.92^{b}	13.87^{a}	7.91^{b}	10.21^{b}	3.79^{ab}	22.31^{a}	6.48^{a}	8.69^{a}	1.22^{a}	0.93^{a}
CV18-1A	63.0^{a}	66.9^{a}	1.81^{c}	14.48^{a}	9.66^{a}	13.09^{a}	2.09^{c}	13.81^{b}	6.21^{a}	6.14^{b}	1.17^{a}	0.66^{b}
! CV (%)	15.66	14.65	41.87	13.42	26.39	21.78	30.78	16.55	21.15	26.65	21.35	26.91
Phosphorus levels (kg/ha)												
0	55.4^{a}	58.9^{a}	3.35^{a}	13.39^{a}	8.39^{a}	10.97^{a}	3.37^{a}	17.79^{a}	5.62^{b}	7.93^{a}	1.06^{b}	0.85^{a}
30	51.9^{a}	55.4^{a}	3.06^{a}	13.23^{a}	8.57^{a}	10.87^{a}	3.19^{a}	17.35^{a}	5.95^{b}	7.91^{a}	1.13^{b}	0.85^{a}
60	53.1^{a}	55.7^{a}	3.39^{a}	13.33^{a}	8.30^{a}	11.06^{a}	3.47^{a}	18.26^{a}	6.74^{a}	7.91^{a}	1.27^{a}	0.85^{a}
90	54.9^{a}	58.3^{a}	3.24^{a}	13.08^{a}	7.97^{a}	10.53^{a}	3.39^{a}	18.38^{a}	5.89^{b}	7.72^{a}	1.11^{b}	0.83^{a}
! CV (%)	15.66	14.65	41.87	13.42	26.39	21.78	30.78	16.55	21.15	26.65	21.35	26.91
Moisture regimes (kPa)												
15	51.7^{b}	55.4^{b}	4.05^{a}	13.69^{a}	9.15^{a}	12.07^{a}	4.19^{a}	17.61^{a}	6.42^{a}	9.47^{a}	1.11^{a}	0.97^{a}
50	52.8^{b}	56.1^{b}	3.14^{b}	13.53^{a}	8.33^{ab}	10.65^{b}	3.26^{b}	18.49^{a}	6.23^{b}	7.27^{b}	1.19^{a}	0.79^{b}
75	56.9^{a}	59.8^{a}	2.59^{b}	12.55^{b}	7.45^{b}	9.84^{b}	2.62^{c}	17.74^{a}	5.50^{c}	6.86^{b}	1.12^{a}	0.77^{b}
! CV (%)	15.66	14.65	41.87	13.42	26.39	21.78	30.78	16.55	21.15	26.65	21.35	26.91

! CV = Coefficient of variation, G = Genotype, P = Phosphorus, M = Moisture regimes, NoDfl = Number of days to flowering, NoDpd = Number of days to pod formation, NoPdpp = Number of pods per plant, PdL = Pod length (cm), NoSdpp = Number of seeds per pod, NoCpp= Number of cavities per pod, SWtpp = Seed weight per plant(g), HSW = Hundred Seed Weight (g), BMfl= Biomass at flowering (g), TBYh =Total biological yield (g), WUEfl = Water use efficiency at flowering (g/mm) and WUEh = Water use efficiency at harvest (g/mm).

4.4.3 Treatment interaction effect of measured phenological and yield attributes

The significant PxM interaction effect recorded the highest number of seeds per plant under well-watered treatment (15 kPa) with 30 kg P ha^{-1} designated by P1M1 (Figure 4.1). However, severe soil moisture stress (75 kPa) with the application of 90 kg P ha^{-1} designated by P3M3 resulted in over 52% reduction in the number of seeds per pod (Figure 4.1A). The highest mean pod length of 14.21 cm obtained with the application of 30 kg P ha^{-1} under well-watered treatment (15 kPa) is statistically comparable to the mean pod length obtained with P3M1 and P2M2 treatments (Figure 4.1B).

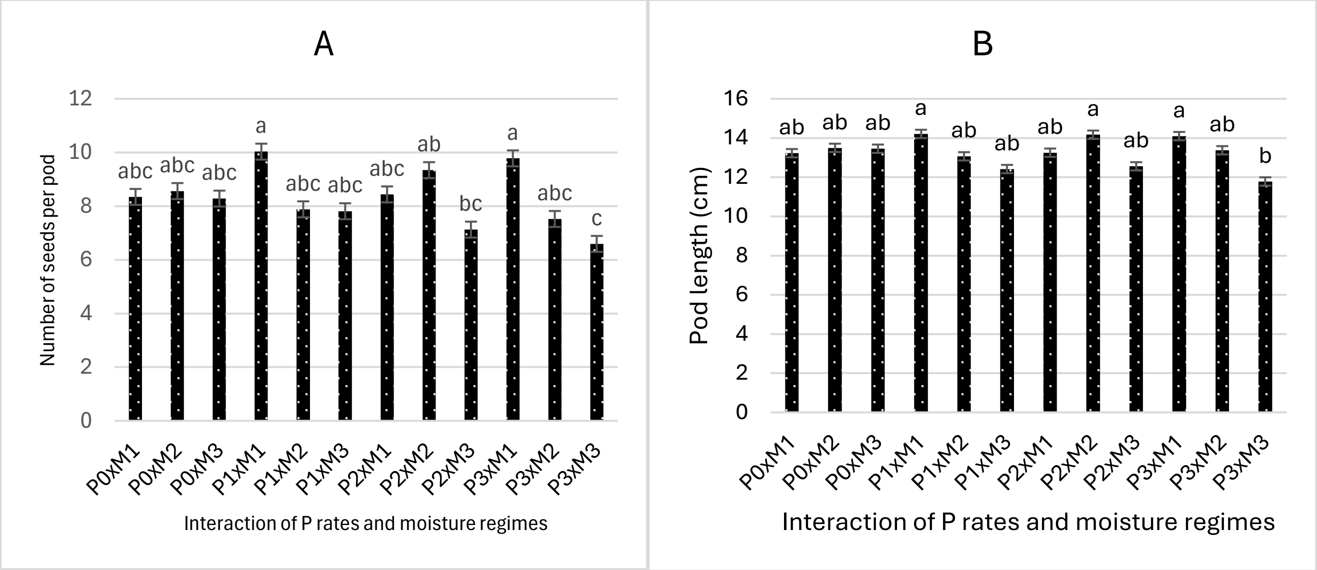


Figure 4.1: Interaction effect of P levels and moisture regimes on the mean number of seeds (A) and pod length (B) per pod of cowpea.

The significant GxM interaction effect recorded the greatest number of pods per plant (6.19) with CV17I genotype irrigated at 15 kPa designated G1M1, although its measured value is, however, statistically comparable to the 5.54 obtained with CV17I irrigated 50 kPa designated G1M2 (Figure 4.2). Conversely, the significant GxP interaction effect on the total biological yield at harvest and WUEh were highest with CV17I fertilized at 60 kg P ha^{-1} (9.66 g/plant) designated G1P2 and CV17B without P fertilization (1.039 g mm^{-1}) designated G3P0, respectively while the CV18-1A genotype fertilized at 90 kg P ha^{-1} designated G4P3 produced the least total biological yield and WUEh of 5.37 g/plant) and 1.039 g mm^{-1}, respectively (Figure 4.3).

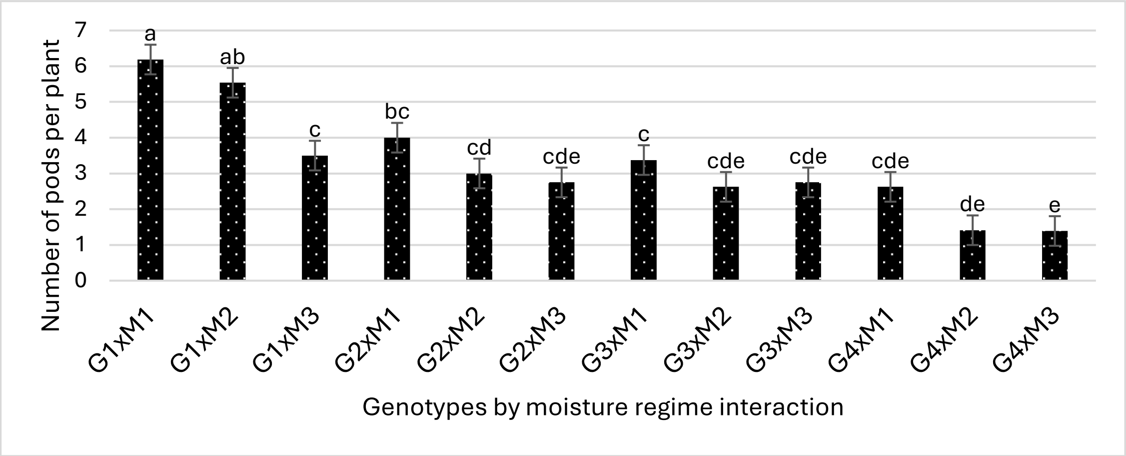


Figure 4.2: Interaction effect of genotypes and moisture regime on the mean number of pods per plant.

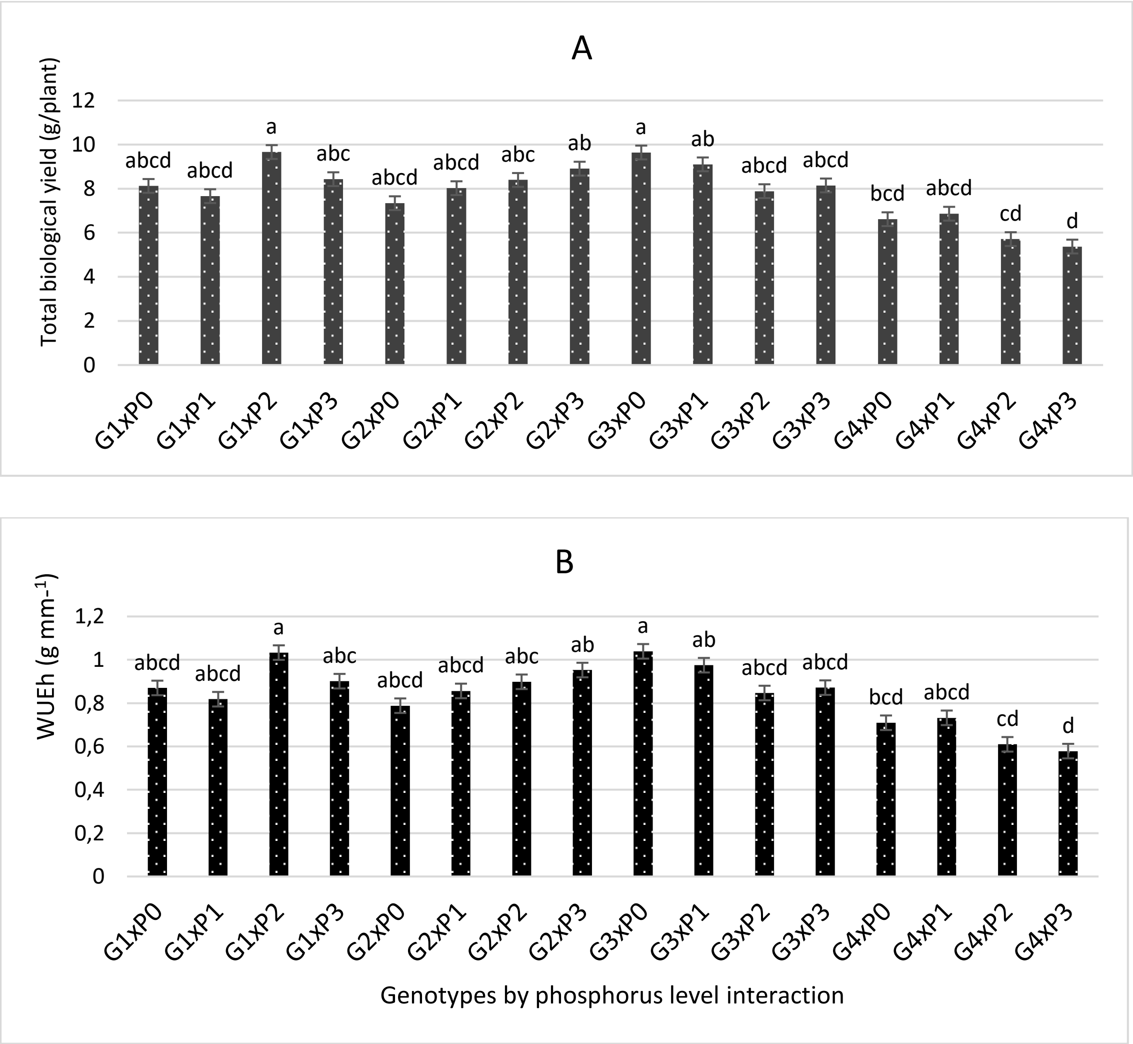


Figure 4.3: Genotypes and P interaction effect on total biological yield (A) and water use efficiency at harvest (B).

4.4.4 Correlation, Regression, PCA, and cluster analyses

The Pearson correlation matrix among all measured phenological and yield parameters as well as computed WUE are presented in Table 4.3. The results revealed a significant ($p \leq 0.05$) relationship among large numbers of the variables, albeit sometimes a very weak and negative relationship. A very highly positive correlation was observed between number of days to pod formation and number of days to flowering, whereas the counted number of pods per plant showed a significant ($p \leq 0.05$) but negative correlation with both number of days to pod formation and number of days to flowering. Similarly, pod length exhibited a moderately significant ($p \leq 0.05$) but negative correlation with number of pods per plant. While the number of seeds per pod displayed a negatively low but significant ($p \leq 0.05$) correlation with the number of pods per plant, it showed a very highly significant ($p = 0.000$) and positive correlation with pod length. Similarly, the number of cavities per pod had a very strong and significant ($p = 0.000$) correlation with both pod length and number of seeds per pod. Surprisingly, seed weight per plant revealed a negatively moderate but very highly significant ($p = 0.000$) correlation with both the number of days to flower and number of days to pod formation; but had a highly significant ($p = 0.000$) and positive correlation with the number of pods per plant.

Interestingly, HSW had significantly ($p = 0.000$) low and negative relationship with the number of days to flowering, number of days to pod formation, number of pods per plant, number of seeds per pod, and number of cavities per pod but displayed significantly ($p = 0.000$) positive effect on the pod length and seed weight per plant. The obtained biomass yield at flowering recorded a negatively significant ($p \leq 0.05$) but weak correlation with only the number of days to flowering whereas the TBYh had a very highly significant ($p = 0.000$) but moderate and negative correlation with both the number of days to flowering and number of days to pod formation. Also, the TBYh had a very strongly significant ($p = 0.000$) strong correlation with the number of pods and seed weight per plant but weak relationship with HSW. The WUEh had a very strong and significant ($p = 0.000$) relationship with the number of pods per plant, seed weight per plant, hundred seed weight and TBYh but weak relationship with the HSW. The relationship between WUEh and each of number of days to flowering and pod formation was significant though moderate and negative.

Table 4.3: Pairwise correlation matrix (r) among cowpea phenotypic and yield attributes

	NoDfl	**NoDpf**	**NoPdpp**	**PdL**	**NoSdpp**	**NoCpp**	**SWtpp**	**HSW**	**BMfl**	**TBYh**	**WUEfl**	**WUEh**
NoDfl	1											
NoDpf	0.951***	1										
NoPdpp	-0.278***	-0.286***	1									
PdL	0.053	0.096	-0.332*	1								
NoSdpp	-0.007	0.044	-0.143*	0.747***	1							
NoCpp	0.085	0.130	-0.041	0.586***	0.737***	1						
SWtpp	-0.372***	-0.361***	0.512***	0.122	0.121	0.089	1					
HSW	-0.208***	-0.236***	-0.165***	0.219***	-0.129	-0.275***	0.380***	1				
BMfl	-0.151*	-0.127	0.022	0.021	0.049	0.133	-0.036	-0.115	1			
TBYh	-0.326***	-0.331***	0.522***	-0.006	0.076	0.098	0.791***	0.177*	0.006	1		
WUEfl	-0.096	-0.085	-0.061	-0.033	-0.031	0.044	-0.177*	-0.12	0.954	-0.126	1	
WUEh	-0.313***	-0.323***	0.505***	-0.035	0.037	0.055	0.764***	0.193*	-0.028	0.991***	-0.124	1

= indicate significant effect at 5% level, **= indicate significant effect at 1% level and *= indicate significant effect at 0.1% level, Number of days to flowering =NoDfl, Number of days to pod formation =NoDpf, Number of pods per plant =NoPdpp, Pod length =PdL, Number of seeds per pod =NoSdpp, Number of cavities per pod =NoCpp, Seed weight per plant =SWtpp, Hundred Seed Weight = Hundred Seed Weight, BMfl= Biomass at flowering, TBYh =Total biological yield, WUEfl = Water use efficiency at flowering and WUEh = Water use efficiency at harvest.*

The results of regression analysis as shown in figures 4.4 & 4.5 below present the quadratic polynomial response curves of the various phenological and yield attributes to P fertilizer rates. The curves reveal weak r^2-value of 0.065 and 0.224, respectively for mean number of pods weight (Figure 4.4) and seed weight (Figure 4.5) per plant. However, strong R^2-value of between 0.655 and 0.679 was observed on the pot length, number of cavities and hundred seed weight (Figures 4.4 and 4.5). Similarly, the mean number of days to flowering, pod formation, mean number of seeds per pod total biological yield and water use efficiency at maturity had strong R^2-values ranging from 0.879 to 0.990 (Figure 4.4 and 4.5).

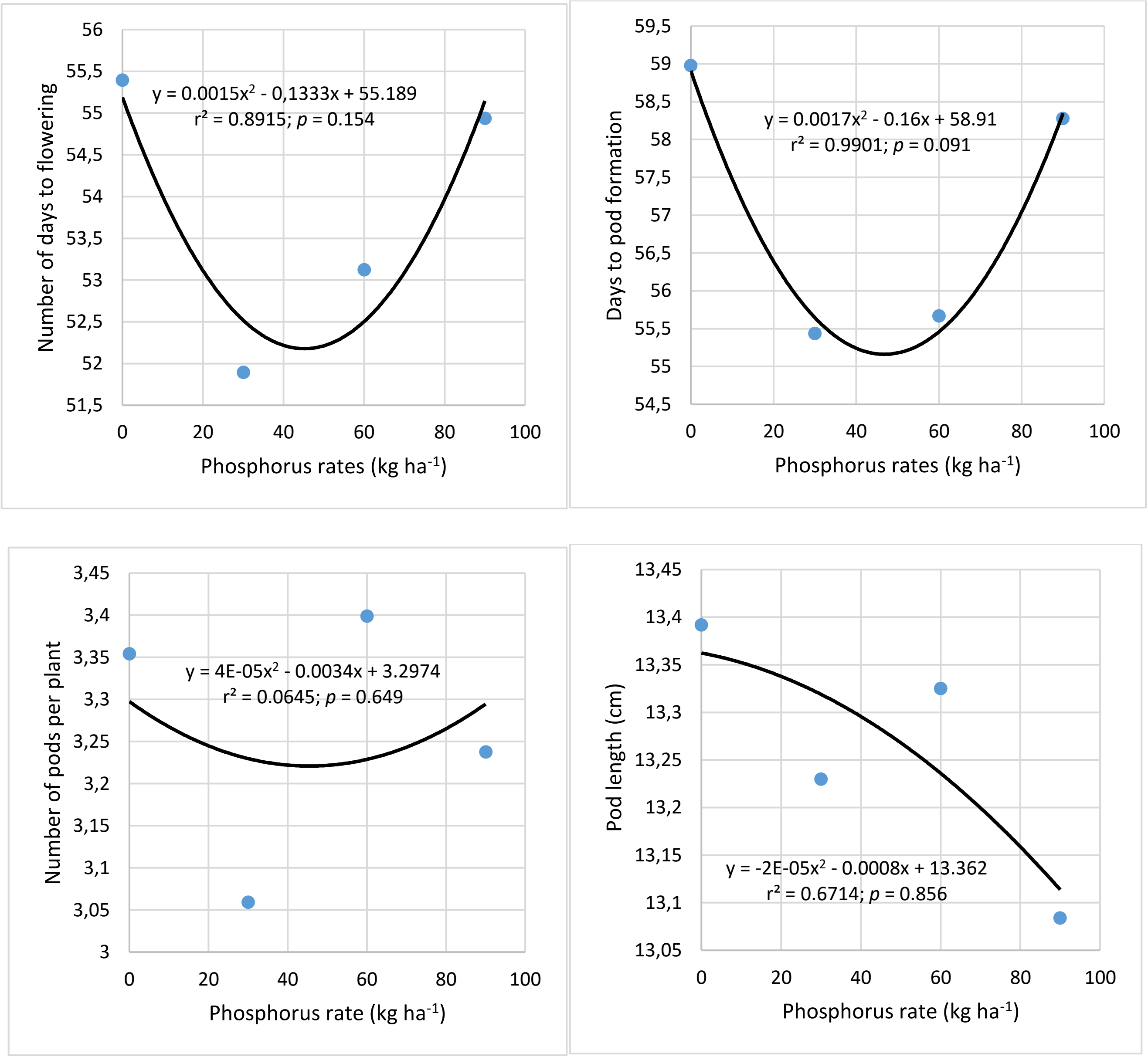
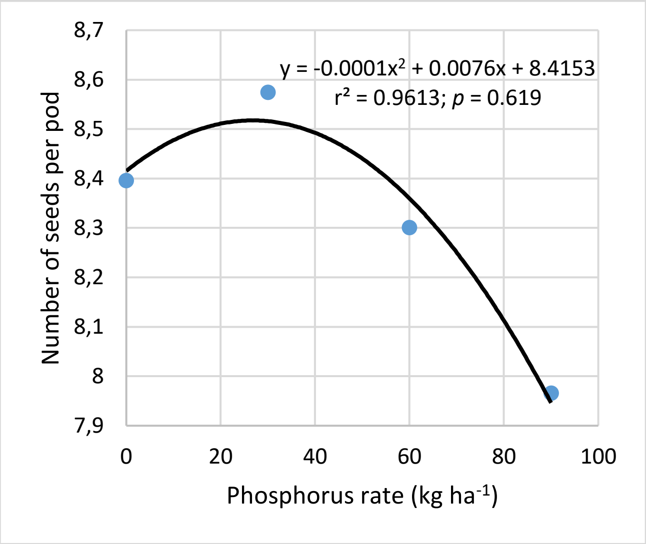
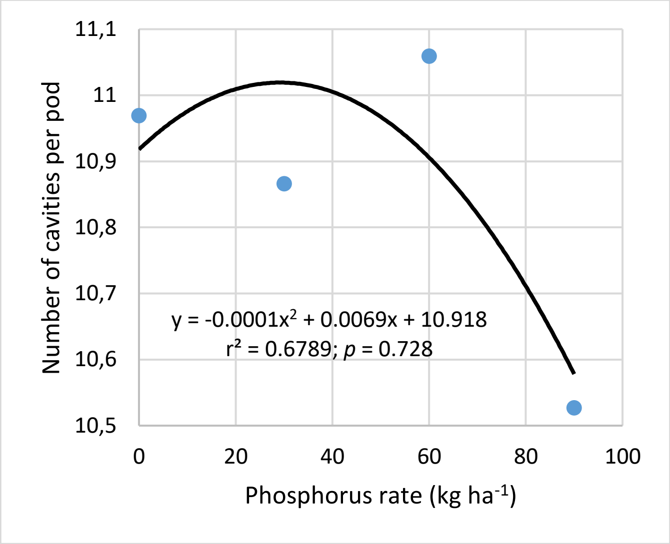
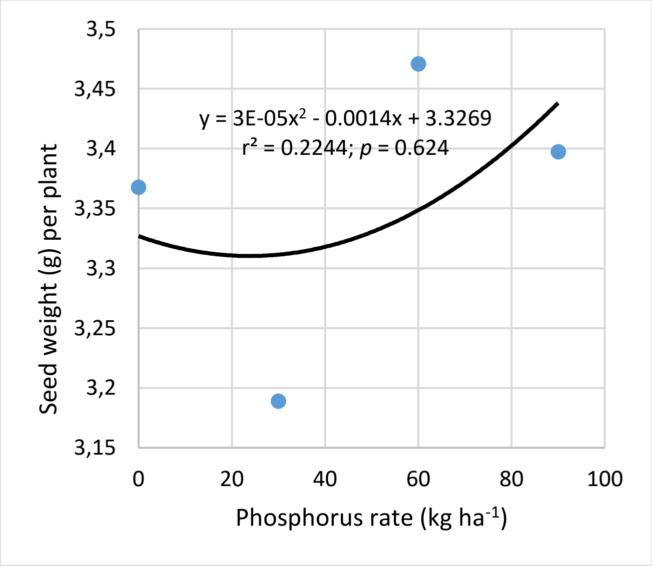
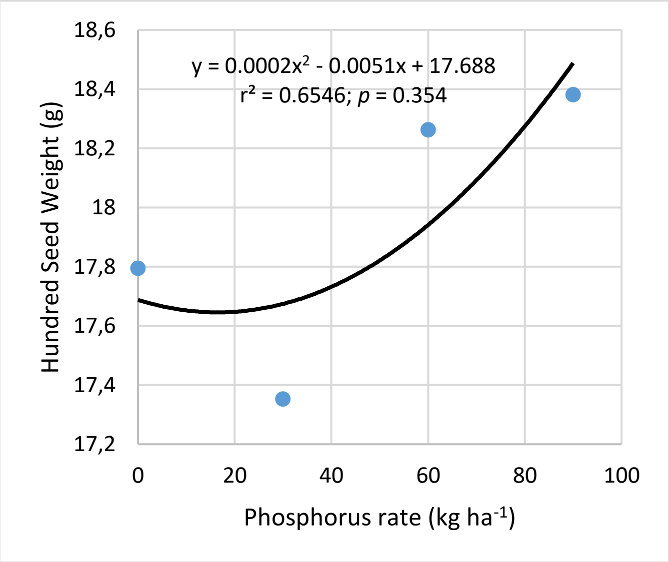


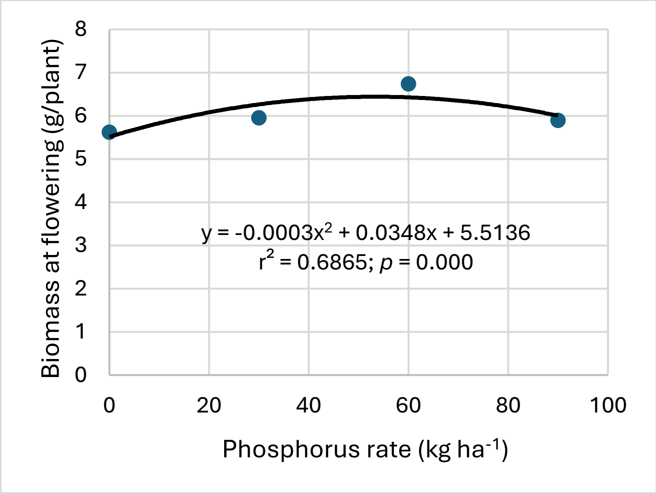
Figure 4.4: Quadratic polynomial of phosphorus fertilizer application rate on phenological and yield attributes.

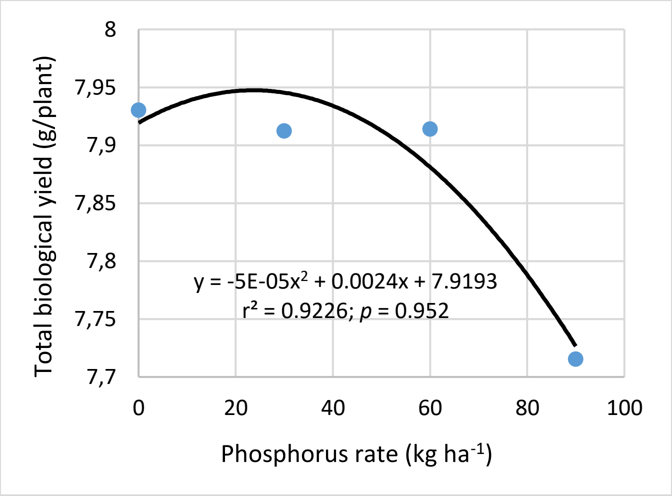

8,7
8,6
8,5
8,4
8,3
8,2
8,1
8
7,9
Number of seeds per pod
y = -0.0001x² + 0.0076x + 8.4153
r² = 0.9613; p = 0.619
0
20
40
60
80
100
Phosphorus rate (kg ha-1)


11,1
11
10,9
10,8
10,7
10,6
10,5
Number of cavities per pod
y = -0.0001x² + 0.0069x + 10.918
r² = 0.6789; p = 0.728
0
20
40
60
80
100
Phosphorus rate (kg ha-1)


3,5
3,45
3,4
3,35
3,3
3,25
3,2
3,15
Seed weight (g) per plant
y = 3E-05x² - 0.0014x + 3.3269
r² = 0.2244; p = 0.624
0
20
40
60
80
100
Phosphorus rate (kg ha-1)


18,6
18,4
18,2
18
17,8
17,6
17,4
17,2
Hundred Seed Weight (g)
y = 0.0002x² - 0.0051x + 17.688
r² = 0.6546; p = 0.354
0
20
40
60
80
100
Phosphorus rate (kg ha-1)


8
7
6
5
4
3
2
1
0
Biomass at flowering (g/plant)
y = -0.0003x² + 0.0348x + 5.5136
r² = 0.6865; p = 0.000
0
20
40
60
80
100
Phosphorus rate (kg ha-1)


8
7,95
7,9
7,85
7,8
7,75
7,7
Total biological yield (g/plant)
y = -5E-05x² + 0.0024x + 7.9193
r² = 0.9226; p = 0.952
0
20
40
60
80
100
Phosphorus rate (kg ha-1)

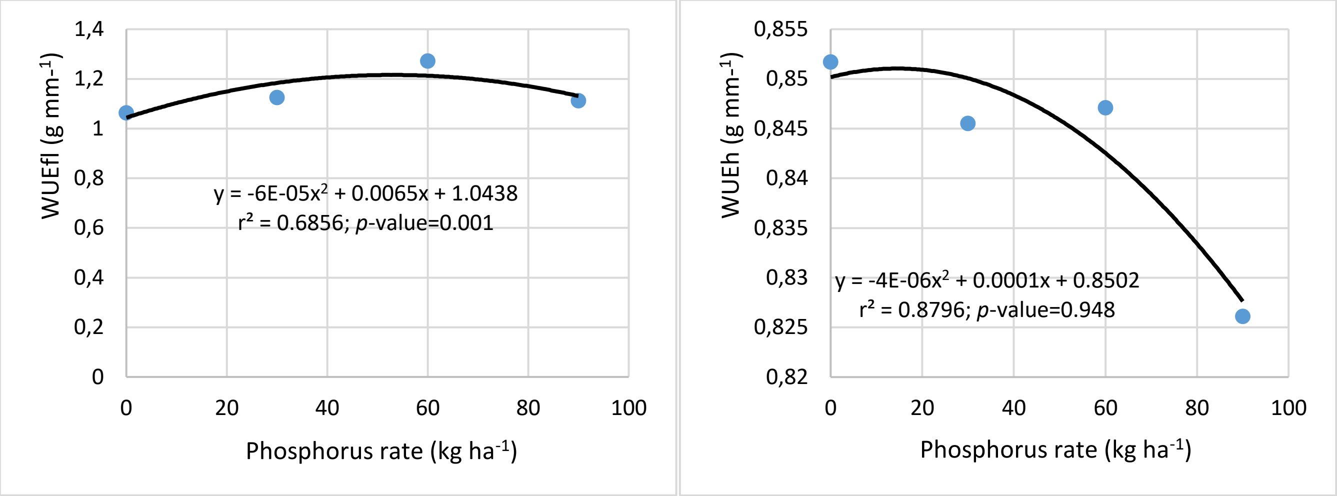


Figure 4.5: Quadratic polynomial of phosphorus fertilizer application rate on yield attribute and water uses efficiency of cowpea.

The results of component loadings from PCA for the twelve cowpea traits measured are presented in Table 4.4 below. The loadings of the measured traits had a relatively high variance for PC1 and PC2 at 32.07 and 18.13%, respectively. Interestingly, the variance and the eigenvalue decrease as the PC number increases. A biplot display from a PCA that visualizes the relationship between the treatment factors and measured phenological and yield and attributes observed in the dataset is depicted in Figure 4.6. The components (C) exist in linear arrangements, with the variables capturing the largest variance in the data. Component 1 captured the most variance compared to C2, which captured the second most order. Interestingly, PdL and HSW were strongly associated with C1, although most traits are between the interception of negative and positive values for C1 (Figure 4.6). The number of days to flowering and number of days to pod formation showed a strong correlation with a very strong association with C2 (Figure 4.6). Similarly to seed weight per plant, TBY and WUEh have a very strong positive relationship, albeit interpolated in C1 and C2.

Table 4.4: Loadings of the measured cowpea traits onto the twelve principal components

Parameter	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC 12**
NoDfl	-0.884	-0.017	-0.177	0.053	0.267	0.207	-0.032	0.232	0.055	-0.042	0.100	0.026
NoDpf	-0.889	-0.002	-0.133	0.049	0.311	0.177	-0.058	0.210	0.018	0.033	-0.104	-0.031
NoPdpp	0.029	0.960	-0.197	0.062	-0.021	0.041	-0.007	-0.005	-0.019	0.176	0.030	-0.012
PdL	-0.102	-0.753	0.310	0.074	0.464	0.029	-0.163	-0.172	-0.187	0.115	0.023	0.002
NoSdpp	-0.237	0.773	0.128	0.092	0.441	0.014	-0.158	-0.257	-0.151	-0.109	-0.014	0.018
NoCpp	-0.393	0.126	0.597	-0.356	0.346	-0.096	0.409	-0.094	0.203	0.023	0.001	0.003
SWtpp	0.662	0.032	-0.203	0.299	0.415	-0.170	0.347	0.231	-0.233	-0.013	-0.003	0.007
HSW	0.419	-0.152	-0.407	0.630	0.311	0.093	0.033	-0.181	0.315	0.001	-0.003	-0.005
BMFl	0.124	0.179	0.725	0.427	0.034	-0.327	-0.262	0.227	0.125	0.005	0.002	0.003
TBYd	0.836	0.088	0.172	-0.324	0.285	0.213	-0.109	0.085	0.036	-0.039	0.028	-0.091
WUEFl	0.028	0.063	0.619	0.486	-0.329	0.473	0.187	-0.011	-0.096	-0.008	-0.003	-0.002
WUEH	0.852	0.066	0.095	-0.328	0.190	0.271	-0.116	0.118	0.082	0.026	-0.031	0.087
Eigenvalue	3.849	2.175	1.712	1.267	1.194	0.573	0.474	0.362	0.290	0.062	0.024	0.018
% variance	32.072	18.127	14.270	10.561	9.953	4.773	3.946	3.015	2.415	0.515	0.202	0.151

PC = principal components, NoDpf = Number of days to flowering, NoDpf = Number of days to pod initiation, NPPP= Number of pods per plant, PdL= Pod length, NoSdpp = Number of seeds per pod, NoCpp =Number of cavities per pod, SWtpp = Seed weight per plant and HSW= Hundred Seed Weight, BMfl= Biomass at flowering, TBYh =Total biological yield, WUEfl = Water use efficiency at flowering and WUEh = Water use efficiency at harvest.

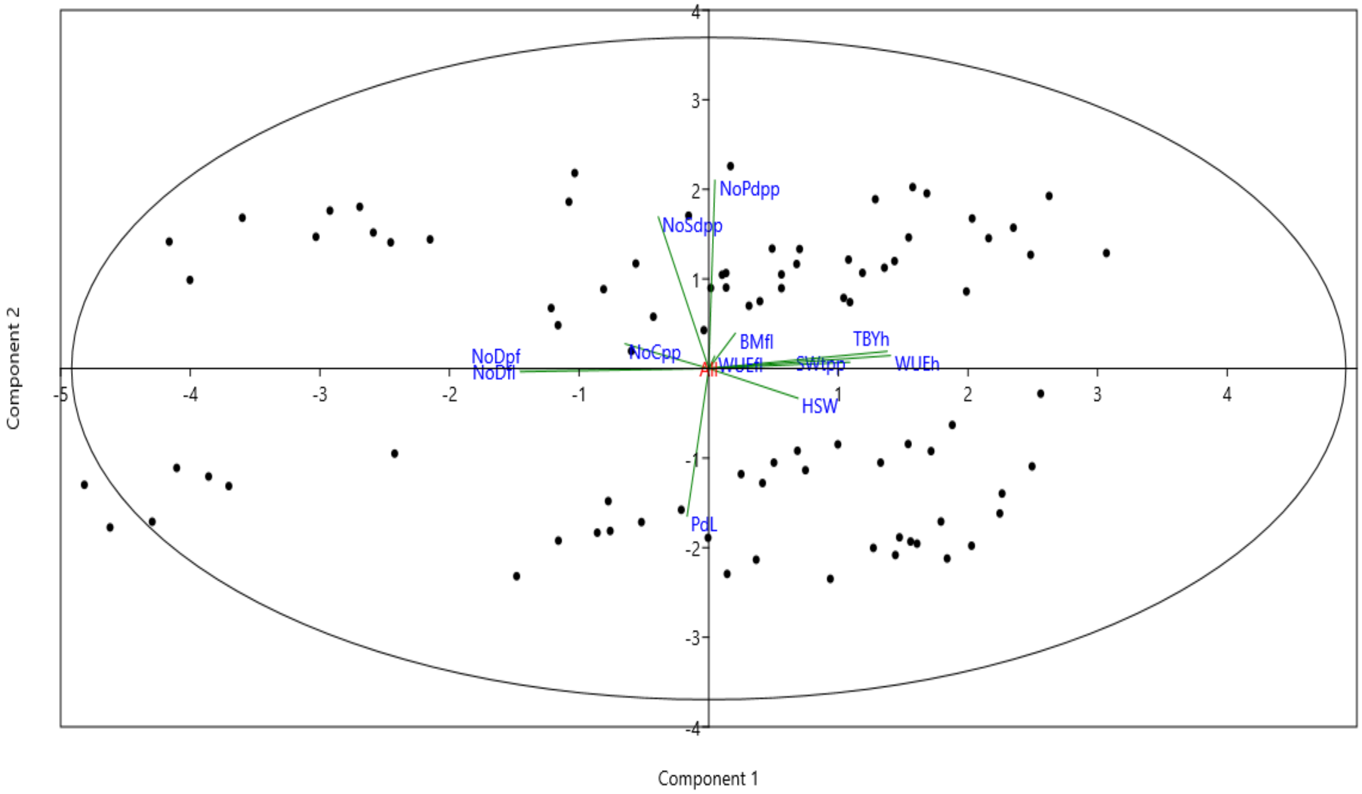


Figure 4.6: Biplot from PCA showing graphical display of the measured phenotypic and yield attributes.

Figure 4.7 depicts a dendrogram arrangement of clusters formed by the hierarchical clustering effect of treatment factors on the assessed phenological and yield attributes. The similarity scale ranges from about 0.975 to 1.000, suggesting that the closer the branches merge towards the top of the dendrogram, the closer to 1.000 are the clusters (Figure 4.7). Short branch lengths indicate high similarity between the clusters, particularly for the two-way interaction leading to the GxPxM combinations having a very close association. Interestingly, despite the close association between GxPxM interaction, CV17I without P addition irrigated at 15 kPa (G1xP0xM1) showed far less association with CV17B fertilized at 60 kg P ha^{-1} under severe soil moisture stress with irrigation at 75 kPa designated by G3xP2xM3 as shown in Figure 4.6. Moreover, the clusters show very high similarity merging close to the 1.000 similarity mark. Likewise, CV17I without P addition irrigated at 75 kPa designated G1xP0xM3 is extremely associated with CV17I fertilized at 90 kg P ha^{-1} under moderate moisture stress (50 kPa) designated by G1xP3xM2. Additionally, the results reveal that there is a clear grouping of treatments (i.e., distinct differences between each group) with early branch separation, suggesting that treatments are quite different (Figure 4.7).

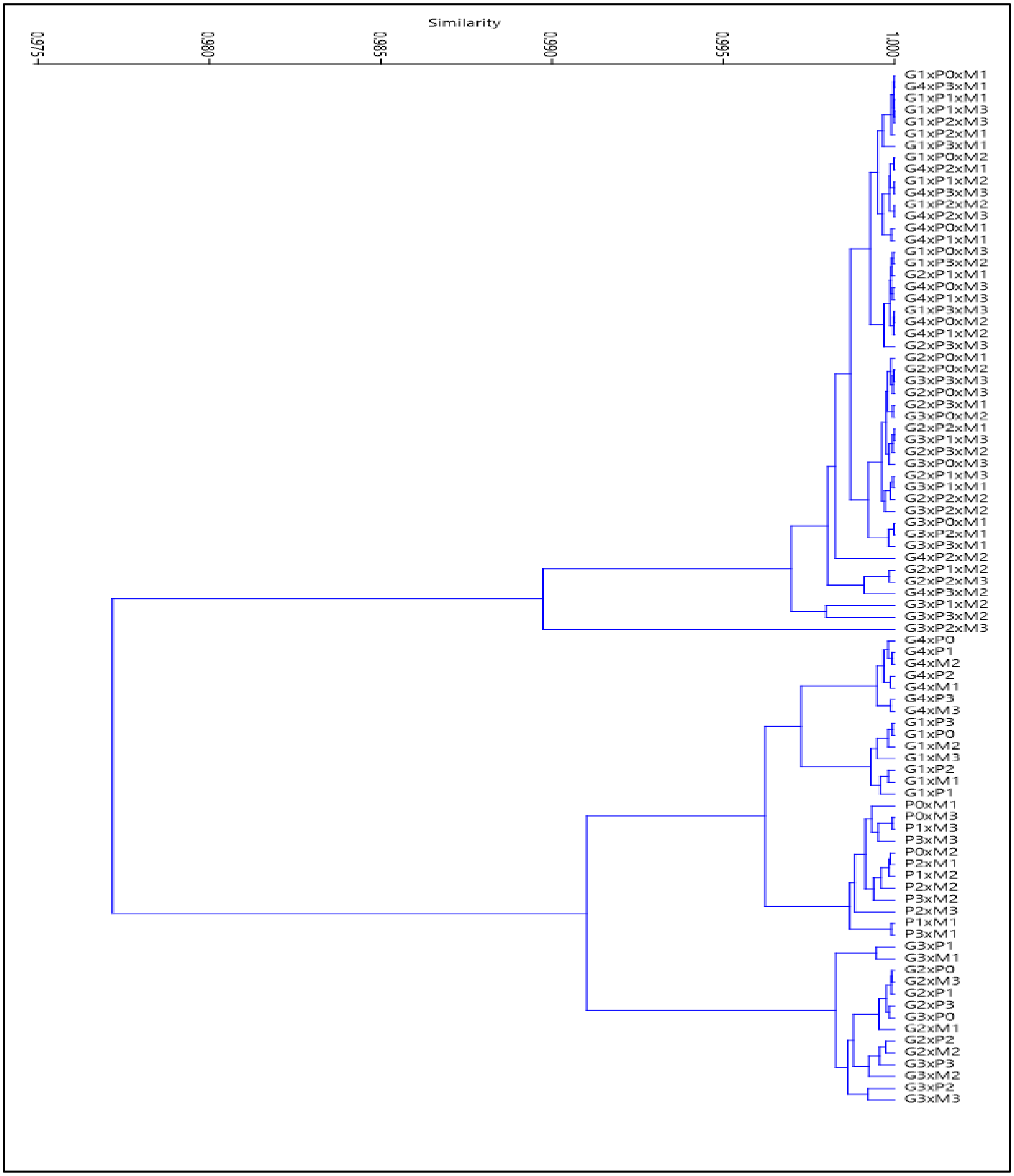


Figure 4.7: Cluster analysis of first and second order treatment interaction effect using group averages and Euclidean distance methods. Genotypes consist of G1 = CV17I, G2 = CV17F, G3 = CV17B and G4 = CV18-1A); phosphorus levels comprised of P0, P1, P2 and P3, which implies 0, 30, 60 and 90 kg ha^{-1}, respectively and moisture regimes M1, M2 and M3 equal to 15, 50 and 75 kPa, respectively.

4.5 Discussion

4.5.1 Main treatments and their interaction on cowpea phenotypic attributes

Number of days to flowering

The number of days to flowering is one of the important phenological trait, that varies widely among plant species due to their genetic makeup. The current study revealed that cowpea genotypes independently affected the number of days to flowering, which is in agreement with the earlier work by Weller and Ortega (2015) who reported that the differences in the genetic makeup of legumes can highly influence the flowering time because of family gene expression. Additionally, Nkhoma *et al.* (2020) consented with the finding that cowpea genotypes had varied flowering thus influencing the maturity dates. These suggest that the cowpea genotypes used in the current study had distinct phenotypic traits that influenced early and late flowering. Moreover, significant effect of the variation in soil moisture regimes on the number of days to flowering suggests that it is a critical factor that influences the timing of flowering, albeit moisture stress often leads to delayed reproductive development as a survival strategy. This observation is consistent with earlier findings by Chen *et al.* (2023) who reported that drought conditions may delay flowering and maturity with plants using adaptive responses aimed to avoid adverse conditions during the critical reproductive phase. Contrarily, Alvarez *et al.* (2018) reported that other crops escape the detrimental effects of drought stress through mechanisms that involve rapid plant development and shortening of the life cycle. The latter is supported by Tekle and Alemu (2016) who reported that early flowering is a best possible escape adaptive mechanism in plants despite having a negative effect on the length of the plant growing period and yield accumulation.

The non-significant G×P×M interaction effect on the mean number of days to flowering suggests that the interaction does not adequately influence flowering time of cowpeas. Similarly, the inconsequential effect of neither G×P nor P×M interaction on flowering date may either suggests that the variation in P levels does not strongly interact with either of both factors (i.e., genotype and moisture) to exert a positive effect on flowering or that the native P level in the soil used was adequate for additional P fertilization to exert further substantial impact on flowering. This finding contradicts the work by Khan *et al.* (2023) who reported that high P levels can mitigate the adverse effects of drought. Moreover, the present study underscores the independent effect of genotype and soil moisture factors on the number of days to flowering. This aligns with Bankole *et al.* (2020) findings that genotypic difference presents a strong association with early and late maturity, which is critical for escaping late-season droughts and fitting into shorter growing seasons. Contrary to the findings of Nadeem *et al.* (2019) who reported that the shortened duration of the reproductive phase facilitates early flowering in legumes, which assists the plants to escape drought during their growth stages and improve their yield component. Nonetheless, the delay in flowering observed in the CV18-1A and 90 kg P ha^{-1} under severe moisture stress combination in this study could be due to the exacerbated stress of high (excess) P application and severe moisture deficit, meaning that the crop could have focused more on vegetative growth other than reproduction.

Number of days to pod formation

The number of days to pod formation is a phenological attribute in most leguminous plants that outline the number of days it takes for the plant to initiate pods, which directly impacts the crop's reproductive and yield success. The non-significant GxP, PxM and G×P×M interaction effects on the number of days to pod formation highlights the complexity of the interaction of P fertilization and moisture regimes on cowpea genotypes, although there is limited research on the second order interaction of the studied factors on number of days to pod formation. Nevertheless, Salim *et al.* (2023), and He *et al.* (2019) reported similar findings that GxP interaction significantly influenced the flowering and podding times. The longest pod initiation time recorded with CV18-1A genotype and 30 kg P ha^{-1} under severe moisture stress regime in the current study aligns with earlier work on soybean by Kodadinne *et al.* (2024) where varied genotypes reportedly exhibit varied responses to moisture stress under water deficit and low P conditions.

4.5.2 Main treatments and their interaction on cowpea yield attributes

Number of pods per plant

The observed significant G×M interaction effect on the mean number of pods per plant in the current study indicates the implication of genotype responses to varying moisture regimes, with similar findings being reported in soybeans by He *et al.* (2017) and chickpea by Naveed *et al.* (2024). The highest number of pods per plant reported in the CV17I genotype irrigated at 15 kPa (well-watered) regime relative to other treatment combinations highlights the empirical genetic effect on cowpea pod production, especially under varying moisture regimes. The reduction in pod production under moisture stress is consistent with plant's survival response of resource allocation to reproductive structures reported by various authors in different crops such as chickpeas (Pushpavalli *et al.,* 2014) and common beans (Tapia *et al.,* 2022). Although numerous studies have shown that P can significantly affect pod and seed production, particularly in P-deficient soils (e.g., Khan *et al.,* 2023; He *et al.,* 2019), results from the present did not allude to this possibly since the soil used had fairly adequate available P level, which may have limited the response to P application. The significant G×M interaction emphasizes the importance of selecting appropriate genotypes for specific moisture regimes to optimize pod production.

Cowpea pod length

Pod length is a critical morphological trait in cowpeas, influencing crop yield. However, the significant P×M interaction effect on cowpea pod length highlights the importance of nutrient and water availability in equally influencing this important morphological trait. The manifested in the highest mean pod length obtained with 30 kg P ha^{-1} application under well-watered treatment (15 kPa). Similar significant interaction effects on pod and seed development have been observed in soybeans by Salim *et al.* (2024). Interestingly, the genotypic variation in pod length reported in the current study with CV18-1A having the longest pod length underscores the role of genetic factors in determining morphological traits and is consistent with previous cowpea research results by Aliyu and Makinde (2016), revealing genotype-yielding response vary depending on a genotype. Hence, the reduction in pod length under moisture stress is associated with the empirical general plant response to drought where plants redirect their energy for survival and optimize productivity as documented for wheat (Khalil *et al.,* 2020).

Number of seeds per pod

The inconsequential effects of GxP and GxPxM interaction on the mean number of seeds produced per pod reported in the current study are coherent finding by Thosago (2015), which highlights that each interaction level of the tested genotypes had no effect suggesting that genotypic variation may have limited the treatment interaction effect on the number of seeds per pod, which might be that some of the studied genotypes are similar. The significant independent effect of genotype and moisture regime on the number of seeds per pod as revealed in the current study underscores the critical role that genetic factors play in determining seed production capacity. Similarly, Zhang *et* *al.* (2024) reported that cowpea genotypes exhibit considerable variation in seed number and size, contrary to the findings of Nkhoma *et al.* (2020), who reported a non-significant effect.

The significant genotypic effect on the mean number of seeds per pod reported in this study is consistent with earlier findings on chickpeas reported by Wang *et al.* (2017). The observed decrease in the mean number of seeds per pod under severe moisture stress aligns with findings reported in chickpeas and soyabean (Pang *et al.*, 2017; Li *et al.*, 2013). Furthermore, He *et al.* (2019) reported that a combination of P and water stress significantly impacted soybean yield components thus informing the need for integrated nutrient and water management strategies. The significant P×M interaction effect on the number of seeds per pod emphasizes the importance of considering these factors in optimizing cowpea seed production. This is consistent with earlier findings reported by Chen *et al.* (2024) who reported that increasing irrigation times and P fertilization indirectly increased seed yield by raising the number of racemes per stem with a crucial role in determining yield components.

Number of cavities per pod

The non-significant G×P×M, G×P, G×M, and P×M interaction effect on the mean number of cavities per cowpea pod suggests a similarity among the various subgroups. In contrast, the significant genotypic effect implies the uniqueness of each genotype, which can be associated with the pod length, suggesting that longer pods might lead to more cavities. Hence, the significantly highest mean number of cavities per pod from the CV18-1A genotype relates to the pod length observed, highlighting the potential high-yielding ability of this genotype. Likewise, the significant effect of the variation in soil moisture regimes on the mean number of cavities per pod underscores the importance of soil moisture conditions in optimizing cowpea grain yield ability through an increased number of pods and cavities for seed filling and protection. Hence, the findings imply that all the cavities have the potential to be filled under adequate moisture conditions, which agrees with previous works by Merchant *et al.* (2022) and Bennett *et al.* (2011).

Seed weight per plant

The observed statistically significant effect of genotypic difference on seed weight per plant attests to the innate unique yielding traits (i.e., genetic makeup and inheritable parental genes) and the possible viability of the studied cowpea genotypes. Similar finding was reported in linseed by Terfa and Gurmu (2020). The significantly highest seed weight per plant obtained with well-watered regime (15 kPa) implies that adequate moisture level is critical to promoting better seed development and overall productivity while the evidently reduced seed weight per plant observed under moisture stress is consistent with several previous studies (Seleiman *et al.*, 2021; Lv *et al.*, 2019; Sehgal *et al.*, 2018). This study's non-significant P effect on the mean seed weight per plant contrasts with the finding in other crops where P application enhances seed and yield traits, particularly in nutrient-poor conditions.

Hundred seed weight

The HSW as a yield component reflects the average seed size and weight, which are key indicators of seed quality. The non-significant G×P×M interaction effect on HSW observed in the present study signifies the lack of robust influential response. The observed significant genotypic variation in HSW underscores the role of genetic factors in seed development and the unique yield traits of the tested genotypes. Both CV17F and CV17B genotypes possess nearly identical HSW and a possible genetic predisposition with larger seeds and more weight relative to CV17I and CV18-1A genotypes with fewer seed weights, suggesting key differences in genetic determinants that affect seed size and weight. Ambika *et al.* (2014) reported a significant interrelation effect of seed size on seed weight. Additionally, similar findings were reported by Danikou *et al.* (2022) in cowpea, mungbean, and soybean genotypes, signifying that precise genotypes exhibit distinct seed size traits with an influential effect on the final seed weight. The observed reduction in HSW due to severe moisture stress imposition at 75 kPa is corroborated by previous results by Sehgal *et al.* (2018) and Zamski (2017) who revealed that drought stress during the initial stage of seed development reduces the seed sink strength by decreasing the number of endosperm cells and amyloplasts formed leading to reduction in seed weight and yield. Although a non-significant outperformance of moderate and severe moisture stress than the adequate moisture regime was observed in terms of HSW, this contradicts findings by Seify *et al.* (2023), possibly due to the prolonged vegetative stage, which delays the reproduction stage under adequate moisture regime.

Biomass yield at flowering and total biological yield at harvest

Above-ground biomass is a key function for grain yield and partitioning of biomass and grains to determine the harvest index, with the flowering stage being a critical time to collect biomass (Dreisigacker *et al.*, 2021). The observed substantial effect of genotype and P fertilization on biomass yield at flowering, with the highest yield obtained with CV17B genotypes, highlights the potential genotypic effect might have on the inheritance of fodder genes that can be very useful as livestock feeds, particularly during the dry season when fodder is scarce, similarly reported by Etana *et al.* (2013) and Gerrano *et al.* (2015). The observed significant biomass yield increase, particularly at 60 kg ha^{-1}, is corroborated by Sun *et al.* (2024), who highlighted that P fertilizer application significantly increased the biomass yield accumulation during the vegetative growth stage. Przywara *et al.* (2023) reported that soil moisture content facilitates biological and chemical transformations for the biomass, thus affecting the above biomass of crops. We observed similar findings in the present study with increasing degrees of moisture stress resulting in increasing biomass yield reduction. This is also supported by Qu *et al.* (2023) findings who reported that severe and long-term drought causes plants to wilt thereby reducing plant biomass.

The aboveground biological yield at harvest encompasses the total biomass generated by plants, which reflects the crops' overall plant growth and health. Thus, total biological yield is a key indicator of crop vitality and effectiveness throughout the growing period (Padhiyar *et al.,* 2017; Murad *et al.,* 2016). The present finding reveals that genotype, moisture regime and interaction between GxP significantly affected the total biological yield. The observed significant genotypic effect on the TBY suggests the varied performance of the different cowpea genotypes evaluated with CV17B showing unique biomass yield at flowering and TBYh, although CV17F showed superior grain (seed) yield advantage as greater biomass may reduce yield (Thapa *et al.,* 2020). Mahajan *et al.* (2018) reported that increasing soil moisture stress negatively affected biomass and seed production in biotypes, which was also the case in the current study as severe moisture stress resulted in 37.5% TBYh reduction compared to the adequate irrigation level (15 kPa). The significant GxP interaction effect on the TBYh and WUEh implies that there is a relatively positive relationship between these two parameters with a possible influence of P addition on the genetic factor response of the tested genotypes. The statistically comparable TBYh for CV17I fertilized at 60 kg P ha^{-1} and CV17B without P fertilized suggests that cowpea growers may opt for either of these genotypes with careful attention to the soil fertilization level.

Water use efficiency at flowering and maturity

Water use efficiency (WUE) is defined as the ratio of biomass to water consumed, which can further outline the amount of carbon integrated as biomass and/or grain produced per unit of water used by the crop (Hatfield and Dold, 2019; Osakabe *et al.,* 2014). Hence, its evaluation of cowpea as a drought-tolerant crop is central to ensuring effective and efficient water resource use. The significant GxP interaction effect of WUE at flowering, as well as genotype, moisture regime, and GxP interaction effect on WUE at harvesting, suggest the major role of the factors in quantifying the WUE at the different stages affecting the crop. He *et al.* (2019) and Jin *et al.* (2015) similarly reported that optimal P application enhanced the water use efficiency, relative water content, and soluble sugars of soybeans. Delpozo *et al.* (2020) reported that genotypes with higher WUE can be more drought-tolerant but may also have lower biomass and grain yield. Remarkably, the observed significantly reduced WUEh due to increasing moisture stress aligns with previous findings by Berger *et al.* (2016), Boutraa *et al.* (2010), and Shangguan *et al.* (2000).

4.5.3 Correlation matrix, Regression, PCA, and cluster analyses among all measured parameters

The observed strong positive correlation between number of days to pod formation and number of days to flowering suggest a harmonised phenotypic relationship between these traits. A similar observation was reported by Kim *et al.* (2020) for soybeans. The weak and negative correlation between pod length and the number of pods per plant suggests a possible influence by partial overlapping of genetic distinct. This observation contradicts earlier findings by Bhushan *et al.* (2007) reported an explicit and significant correlation between the number of pods per plant and pod length. The strong and positive correlation observed between pod length and the number of seeds per pod was expected since longer pods have the potential to accommodate more seeds, which agrees with previous findings reported by Redmon *et al.* (2000) in scotch broom. The observed range r^2-value of between 0.665 and 0.990 based on quadratic analysis highlights a very strong coefficient of determination within the model and represents the best fit in describing the various phenological and yield attributes.

El-Mohsen (2013) reported that when comparing models with the same number of parameters, selecting the model with the highest r^2-value is recommended since higher values indicate that the model accounts for more of the variation in the response variable. For instance, a relatively high R^2 value greater than 0.80 accounts for 80% of most phenological and yield components. Studies by Nazir *et al*. (2021); Skakun *et al*. (2019) similarly reported a considerably high r^2-value of 0.80, indicating a reliable high model fit for the phenological and yield data. Despite the non-linear relationships between crop phenological and yield attributes. However, the physiological and yield response of the crop to fertility levels and soil moisture followed a non-linear sequence due to physiological thresholds beyond which plant responses changed (Ye *et al.*, 2024). Additionally, the high r²-values observed in this study suggest that a quadratic model effectively represents a strong and significant non-leaner response. The strong fit observed in this study supports predictive modeling effective P application and moisture management, thus assisting in developing decision systems for farmers based on phenological and yield predictions related to improving resource use efficiency. On the other hand, the cluster plot in a study by Saroj *et al.* (2021) found that the Indian oilseed crop, *Brassica juncea* (L.) Czern & Coss (AABB) genotypes with taller plants tend to have higher biological yield per plot, suggesting that the tested genotypes in the present study might have genes with tall plant height and higher biomass yield. Similar observations from cluster analysis reported by Seify *et al.* (2023) indicated that moisture stress imposed on genotypic factors with major similarities closer to 1.000 similarity translates to 100%.

4.6 Conclusion

Observations of this study affirm the existence of significant differences among selected measured parameters as affected by the tested genotypes and soil moisture regimes, underscoring the importance of the genetic composition of the various cowpea genotypes. CV17B and CV18-1A are the best-performing genotypes based on the assessed phenological and yield attributes. Although CV18-1A takes a much longer time to flower and initiate pods, it possesses unique attributes such as long pods, the highest number of cavities and seeds per pod, albeit light in weight. The CV17B had the highest HSW, biomass, and WUE at both flowering and harvest. The relatively higher biomass yields recorded in CV17B indicate its suitability as a source of leafy vegetables (i.e., "*Morogo"*) for most smallholder farmers in rural areas, while the grain yield advantage of CV18-1A suggests its suitability for grain production notwithstanding the small seed size and weight. These identified traits underscore the great potential for improvement of the genotypes through modern breeding techniques to enhance cowpea adaptability and productivity. Notwithstanding the drought tolerant ability of cowpeas, their exposure to severe drought conditions resulted in a delayed number of days to flowering and pod initiation, reduced seed yield and WUEh, and overall productivity. The application of variable P fertilizer rates in the current study did not strongly interact with genotype and soil moisture regimes to exert a positive effect on most of the measured cowpea parameters. Additionally, the study recognized genotype selection as the first factor to consider for optimal cowpea production, as an inappropriate genotype may result in significant yield loss even with adequate moisture and optimal P fertilization. These underscore the importance of integrated agronomic management practices, such as selecting appropriate genotypes and effective irrigation to promote high cowpea grain yield. Moreover, the results also revealed that increasing P fertilization beyond the threshold is undesirable for cowpea phenological and yield traits.

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CHAPTER 5

Application of variable phosphorus fertilizer rates under different soil moisture conditions affect cowpea grain mineral, protein, and secondary metabolite compositions

Abstract

Although grain legumes represent a vital plant-protein source, inappropriate agronomic practices and poor soil nutrient management contribute to malnutrition due to a direct link to grain crops' nutrition and protein content. Cowpea plants often experience both biotic and abiotic stressors that may directly affect the formation of secondary metabolites, protein, and nutritional composition. Hence, this study assessed the effect of variation in cowpea genotypes, phosphorus (P) fertilization levels, and soil moisture regimes, including their interaction on selected secondary metabolites, protein, and mineral composition of cowpea grains. A factorial greenhouse fitted into a nested completely randomized design (CRD) was established under a controlled environment comprised of four cowpea genotypes (CV17I, CV17B, CV17F, and CV18-1A), four P levels (0, 30, 60 and 90 kg P ha^{-1}), and three moisture regimes namely well-watered, moderate watered and severe stress regimes representing 15, 50 and 75 kPa, respectively were imposed at the onset of the reproductive stage for 20 days. The harvested grain was assayed for flavonoid, total soluble sugar, protein content, anthocyanin, iron, zinc, and P concentration. Results obtained revealed that all main treatment factors and their interaction exerted significant ($p \leq 0.05$) effects on flavonoid, anthocyanin, iron, zinc, and P contents. Similarly, moisture stress imposition and the GxP, PxM, and GxPxM interaction exerted significant ($p \leq 0.05$) effects on the total soluble sugars. Although there was no significant ($p \geq 0.05$) variation in the protein content among the selected genotypes, it was significantly impacted by the variation in soil moisture content. The study highlights the impact of soil moisture variation as a critical factor that can impact the protein, total soluble sugar, and secondary metabolite contents of cowpea grains.

Keywords: grain quality, grain metabolites, nutrition, soil fertility, and drought

5.1 Introduction

The problems of food insecurity and malnutrition are considered key global health challenges, particularly in many African countries where drought, land degradation, and war are prevalent.

The situation is exacerbated by deficiencies and imbalances in an individual's food intake for energy and nutrients. According to May (2023), the daily protein intake for humans should be about 0.75 to 1.6 g kg^{-1}. However, individuals who live in poverty remain at higher risk of experiencing protein deficiency and malnutrition. Approximately 1.1 billion of the world's poor population reside in the semi-arid tropics, with over 80% of them located in sub-Saharan Africa and South Asia (Evans, 2023). This has directly or indirectly compromised the global nutritional well-being through the nutritional quality of crops produced and the ultimate impact on human nutrition. Robustly, both biotic and abiotic stress destructively affect crop production and cause a profound decrease in annual crop yield and quality. Kamdi *et al.* (2020) reported that about 80% of the world's physical agricultural area is dependent on rainfed irrigation, including 60% under staple food production, thus, highlighting the significance of improved cultivation practices to fulfill global food and nutrition demand. However, soil nutrient deficiencies due to improper fertilizer use and water and poor land management are the primary factors for the lower crop yield and low-quality food grains lacking in nutrition (Kamdi *et al.,* 2024).

Major macronutrients such as N and P are commonly used in most croplands to address deficiencies. However, limited attention is paid to identifying and quantifying the correct measures for secondary and micronutrient deficiencies in different crop production systems, including cowpeas. Chen *et al.* (2021) reported that the management practices of soil nutrients through applying the recommended application rate not only increase crop growth and yield but could also enhance the quality of agricultural products. Nitrogen and P are often the most deficient soil nutrients for crop yield in many parts of the world as these often restrict the absorption of macro- and micronutrients, thus impacting quality and yield. Similarly, extreme drought and temperature stressors affect physiological and morphological attributes by hindering the functional groups of important enzyme molecules, polynucleotides, transport systems for substantial ions and nutrients, and plants' growth and metabolic activities (Hura *et al.,* 2022).

While primary metabolites support plant growth and development, secondary metabolites like phenolic acids and flavonoids are produced in response to stress. Gharibi *et al.* (2019) found polyphenolic compounds in *Achillea pachycephala* are linked to changes in genetic expression during drought. Similarly, Salam *et al.* (2023) reported that flavonoids, such as quercetin and rutin, increased under drought and waterlogging but decreased in sensitive genotypes. Hence, while polyphenols assist in maintaining osmotic potential through free radicals that influence nutrient availability, flavonoids are crucial for antioxidant activity under stress. Hence, metabolomics and mineral composition are valuable tools for crop improvement, thus highlighting the necessity to study abiotic stress tolerance, including the low soil moisture and P conditions effect on plant growth, nutrition, metabolites, and mineral composition. This study assessed the effect of varying genotypes, P fertilization levels, and soil moisture regimes, including their interaction on cowpea grain mineral, protein, and secondary metabolite compositions.

5.2 Methodology

5.2.1 Description of the greenhouse trial

The detailed descriptions of the material and methods of the greenhouse trial and trial layout are as previously explained under sections 3.2.1 to 3.2.4 in chapter 3.

5.2.2 Sample preparation

The harvested seeds from the greenhouse experiment referred to in 5.2.1 were threshed and bulked based on treatment in preparation of milling (Figure 5.1). The bulked seeds were ground using a SM300 milling machine containing 0.1 mm sieve with special precautions to avoid contamination across the different treatments. Each of the fine-grounded powders based on the treatments was transferred to well-labeled plastic zip bags for storage and later analysis.

Figure 5.1: Cowpea seeds harvested from the greenhouse experiment before (A) and after milling (B).

5.3 Data collection

5.3.1 Anthocyanin determination

A pH differential method described by Lee *et al.* (2005) was followed to determine the anthocyanin of the grain, where 1 g of the milled sample was weighed mixed with 9 ml of acidified acetone solvent of 70:29.5:0.5 as acetone: water: acetic acid. The mixture was thoroughly blended and then spun in a centrifuge at 4000 rpm for 20 minutes at 4°C. The liquid supernatant was removed and the remaining solid residue subjected to the same extraction process one more time under the same conditions. The combined extracts were diluted with a buffer solution made from 0.025 M potassium chloride (pH 1.0) and 0.4 M sodium acetate (pH 4.5) solution at a ratio of 1:5 with both buffers adjusted using HCl to pH level of 1.0 and 4.5 respectively. The absorbance of all the samples was measured at two wavelengths of 520 and 710 nm using a visible Spectrophotometer (VWR UV-6300PC) with a double-beam optical system. The anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents (mg/L), was thereafter calculated using the following equation as described by Lee *et al.* (2005):

$$Anthocyanin\ pigment\ (cyanidin - 3 - glucoside\ equivalents, mg/L) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

(Equation 5.1)

Where: A = ($A_{520nm} - A_{700nm}$) pH 1.0 – ($A_{520nm} - A_{700nm}$) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; 1 = pathlength in cm; ε= 26 900 molar extinction coefficients, in L x mol $^{-1}$ and cm^{-1}, for cyd-3-glu; and 10^3 = conversion factor from g to mg.

5.3.2 Flavonoids determination

The determination of the flavonoid concentration in the seeds was according to the method described by Makoi *et al.* (2010). It involves weighing 1 g milled seed sample per treatment and 9 ml of acidified methanol at a ratio of 79: 20: 1 (MeOH: H_2O: HCl) added. The mixture was incubated for 72 hours in a dark place in the laboratory prior to auto-extraction, which was then followed by centrifuging the mixture at 4000 rpm for 20 minutes at 4 °C using a Rotanta 460 automated centrifuge. Thereafter, the absorbance of the clear supernatant liquid was analysed spectrometrically at 300 nm using a double-beam spectrophotometer, and the observed values were expressed in Abs Gdm^{-1}.

5.3.3 Total soluble sugar determination

The method described by Al-Amri (2023) was used for the determination of total soluble sugar (TSS). A subsample of 1 g from the milled seeds was weighed and placed into 50 ml microcentrifuge tubes. Approximately 40 ml of 80% ethanol was added to each of the weighed samples, the mixture was allowed to homogenize for 1 minute, and the tubes were subsequently placed in a water bath at 80° for 20 minutes. Thereafter, the tubes were allowed to cool down at room temperature, and the tubes were subsequently spun in a centrifuge at 6000 rpm for 10 minutes to achieve a clear liquid supernatant. The total soluble sugar concentration was measured using the digital handheld refractometer (Model 95200-002) with a single drop of the liquid supernatant carefully placed on the prism of a refractometer using a pipette and thereafter recorded in $^{O}Brix$.

5.3.4 Total protein content analysis

The Kjeldahl method, as cited by Wanjiku *et al.* (2023), was used to estimate the total protein content, which involves breaking down the sample to release N to be used to estimate the available protein content. A sample of 0.5 g of the dry powder sample was weighed using a digital weighing balance and mixed with 1 g of catalyst mixture of potassium sulphate and copper sulphate (Kjeldahl tablet) into a Kjeldahl tube. About 15 ml of concentrated sulfuric acid was carefully added to the tube. The tubes were placed in a digestion apparatus and heated at 420°C for about 3 hours until the solution became clear. The tubes were then allowed to cool down, and 50 ml of distilled water was added. A blank sample was also processed in the same way without any sample. In the distillation process, 10 ml of 1% boric acid and 2 drops of the bromocresol green indicator were added to a 250 ml conical flask.

The Kjeldahl tube containing the digested sample and the conical flask were connected to preheated distillation equipment. The sample was distilled until the volume of the distillate in the receiving flask reached 40 ml for 5 minutes. The flask containing boric acid and distillate was then removed and prepared for titration. The NH_3 trapped in the boric acid in the distillation flask was titrated with 0.01 of hydrochloric acid (HCl). The protein content (%) was calculated according to Wanjiku *et al.* (2023) using the following formula:

$$\text{Protein content (\%)} = \frac{Titre\ value \times Normality\ of\ acid \times 1.4007 \times 6.25}{Weight\ of\ sample\ taken} \times 100 \qquad \text{(Equation 5.2)}$$

Where: *M* = molarity of the acid, *W* = weight of test portion, *6.25* = conversion factor for protein.

5.3.5 Zinc and iron determination

The grain samples, as prepared under 5.2.2, were used for mineral analyses following methodology described by Ryan *et al.* (2001) in a soil and plant analysis laboratory manual at the Agricultural Research Council Soil, Water and Climate analytical laboratory, Pretoria, South Africa. A sample of 0.5 g seed powder was weighed to a 25 cm^3 calibrated tube added with 70% perchloric acid and 4 ml of 55% nitric acid and thereafter allowed to stay overnight for extraction. After extraction, samples were digested using a digesting block for 2 hours at 100 °C until the samples were completely transparent. The overnight cooling process allowed de-ionized water to fill up to the 25 cm^3 mark. Three standard solutions for each element, including the blank, were prepared for the reading of the concentration of all elements in each digested sample. The concentration of Ca, Zn, Cu, and Fe in the digests was measured using an atomic absorption spectrophotometer (Spectra AA 300). The sample extraction factor of 50 was included in the standards (25 cm^3/0.5 g (v/m ratio) = 50). A calibration graph was created to correlate absorbance with the concentration of nutrients in ppm. The concentration of the sample was obtained directly from the calibration graph in ppm, and the outcomes for Zn and Fe were expressed in mg/kg.

5.3.6 Determination of P content using the colorimetric method

Standard solutions of 0, 10, 20, 30, 40 and 60 ppm of P were prepared according to King (1932) cited by Usoro *et al.* (2010). The sample dilution factor of 50 was included in the standards. Using an automated P analysis, the sample containing reagents comprising Canada-molybdate and stannous chloride solution was analyzed through the system for 30 minutes with a set baseline. The determination of P was based on colorimetric method in which a blue colour was formed by the reaction of ortho-phosphate and the molybdate ion. The phospho-molybdenum complex read at 660 nm for P measurement, and results were reported in percent.

5.4 Data analysis

A detailed description of the study's statistical analysis is outlined in chapter 3, number 3.3. The circles Pearson correlation matrix plot was performed using PAleontological STatistics (PAST) statistical software version 4.03.

5.5 Results

5.5.1 Results of *p*-values for all the measured parameters

A table of *p*-values from the analysis of variance (ANOVA) of metabolite nutrition and mineral content of grain cowpeas is shown in Table 5.1. The results reveal that the various tested genotypes and P application levels exerted a significant ($p \leq 0.05$) effect on the flavonoid, anthocyanin, grain P, Fe, and Zn content with inconsequential ($p \geq 0.05$) effect on the TSS and protein content. On the other hand, the variation in soil moisture content exerted a significant ($p \leq 0.01$) effect on all assessed parameters, including P, Fe, and Zn content. Equally, the GxP interaction significantly improved all the assessed grain quality parameters. Although the genotypes x moisture regimes interaction significantly ($p \leq 0.05$) affected the content of flavonoid, anthocyanin, P, Fe, and Zn content, the TSS and protein contents were not significantly affected. Likewise, phosphorus x moisture regimes interaction had a positive ($p \leq 0.05$) effect on all measured quality parameters except protein. The second-order GxPxM interaction also exerted a significant ($p \leq 0.01$) effect on all assessed quality parameters except for the protein content.

Table 5.1: *p*-values for phenological and yield attributes of cowpea

S of V	Flavonoid	TSS	ACN	Protein	Phosphorus	Iron	Zinc
G	0.000***	0.787^{ns}	0.000***	0.907^{ns}	0.000***	0.000***	0.000***
P	0.000***	0.230^{ns}	0.000***	0.820^{ns}	0.000***	0.000***	0.000***
M	0.000***	0.000***	0.000***	0.004**	0.000***	0.000***	0.000***
GxP	0.000***	0.009**	0.000***	0.487^{ns}	0.000***	0.000***	0.000***
GxM	0.000***	0.276^{ns}	0.000***	0.463^{ns}	0.000***	0.000***	0.000***
PxM	0.000***	0.000***	0.000***	0.749^{ns}	0.000***	0.000***	0.000***
GxPxM	0.000***	0.009**	0.000***	0.777^{ns}	0.000***	0.000***	0.000***

*S of V implies sources of variation, G = Genotype, P = Phosphorus levels, M= Moisture regimes, ns = not significant; *, ** and *** indicates significant effect of treatment at 5%, 1% and 0.1%, respectively; TSS = Total Soluble Solids and ACN = Anthocyanin.*

5.5.2 Main treatment effects on the measured contents of secondary metabolite, protein and mineral contents of grain cowpea

The findings of the present study reveal CV17F genotype had a relatively high flavonoid content of 2.19 mg/g dm, which is about 10% higher compared to that of CV17I and CV18-1A genotypes with a lower content of 1.97 mg/g dm (Table 5.2). Interestingly, the control treatment without P addition had a significantly highest flavonoid content, despite an inadequate range between 2.04 and 2.06 mg/g dm under 30, 60, and 90 kg P ha^{-1} fertilization levels. Introducing severe soil moisture stress at 75 kPa significantly reduced flavonoid content by about 10.96% compared to a well-watered regime at 15 kPa. Additionally, the results revealed a significant increase in the TSS under severe water stress (75 kPa) conditions, accounting for 19.71^{O}Brix (Table 5.2). While CV17I, CV17F and CV17B genotypes gave a statistically similar value ranging between 0.609 and 0.649 mg/g dm (Table 5.2). The significant effect of moisture composition on protein content ultimately decreased with a decreasing moisture level with severe moisture stress significantly reduced the protein content by 20%. CV18-1A genotype attributed to the highest P and Zn content. The significant effect of 60 kg P ha^{-1} substantially increased the P and Fe content in grain. Similarly, a significantly higher Zn content was observed under the control without any P addition. Interestingly, the imposition of moisture at 50 kPa significantly increased the Fe and Zn, while the highest P content was observed under the well-watered regime irrigated at 15 kPa.

Table 5.2: Single factor effect genotypes, P levels and Moisture regimes on mineral, nutritional, and secondary metabolites of cowpea grain

TMF	**Flv-2**	**TSS**	**ACN**	**Pro**	**%P**	**Fe**	**Zn**
Cowpea genotypes							
CV17I	1.97^{b}	18.78^{a}	0.649^{a}	27.14^{a}	0.525^{b}	138.02^{a}	37.10^{d}
CV17F	2.19^{a}	19.15^{a}	0.614^{a}	26.35^{a}	0.485^{d}	74.77^{b}	42.40^{c}
CV17B	2.16^{a}	18.98^{a}	0.609^{a}	26.61^{a}	0.490^{c}	70.48^{c}	42.81^{b}
CV18-1A	1.97^{b}	19.02^{a}	0.377^{b}	25.65^{a}	0.529^{a}	68.27^{d}	48.08^{a}
! CV (%)	3.73	8.32	20.32	26.72	0.64	1.08	0.75
Phosphorus levels (kg/ha)							
0	2.15^{a}	19.00^{a}	0.466^{b}	25.48^{a}	0.508^{b}	74.88^{b}	43.48^{a}
30	2.04^{b}	18.86^{a}	0.661^{a}	26.61^{a}	0.508^{b}	66.31^{d}	42.00^{c}
60	2.06^{b}	19.41^{a}	0.597^{a}	26.26^{a}	0.518^{a}	137.79^{a}	42.89^{b}
90	2.05^{b}	18.66^{a}	0.524^{b}	27.40^{a}	0.496^{c}	72.56^{c}	42.04^{c}
! CV (%)	3.73	8.32	20.32	26.72	0.64	1.08	0.75
Moisture regimes (kPa)							
15	2.19^{a}	18.84^{b}	0.431^{b}	27.64^{a}	0.521^{a}	67.42^{c}	42.20^{b}
50	2.07^{b}	18.40^{b}	0.655^{a}	28.76^{a}	0.503^{b}	122.48^{a}	43.48^{a}
75	1.95^{c}	19.71^{a}	0.600^{a}	22.92^{b}	0.498^{c}	73.75^{b}	42.13^{b}
! CV (%)	3.73	8.32	20.32	26.72	0.64	1.08	0.75

TMF = Treatment factors, TSS= Total Soluble Solids (^{O}*Brix), Pro=Protein (%), Flv-2= Flavonoid (mg/g dm), ACN = Anthocyanin (mg/g dm), %P = Phosphorus (%), Zn = Zinc (mg/kg), Fe = Iron (mg/kg), and ! CV = Coefficient of variation.*

5.5.3 First-order treatment interaction effect on cowpea secondary metabolites, protein, and mineral composition

Table 5.3 outlines the mean values of GxP interaction effect on the assessed secondary metabolites, protein and mineral composition of cowpea grain. The CV17F genotype fertilized at 90 kg P ha^{-1} produced cowpea grains with the highest flavonoid concentration while CV18-1A fertilized at 90 kg P ha^{-1} recorded the least flavonoid concentration. Notably, CV18-1A genotype without P addition had cowpea grains with the highest mineral P and Zn content while CV17I genotype fertilized at 60 kg P ha^{-1} had grains with the highest mineral Fe content. Furthermore, seeds from CV17B genotype fertilized at 30 kg P ha^{-1} gave the highest anthocyanin content while seeds from CV18-1A without P addition had the least anthocyanin content.

The CV18-1A genotype irrigated at 15 kPa recorded 33% higher flavonoid content compared to the same genotype exposed to moisture stress with irrigation at 75 kPa where the concentration recorded was 1.61 mg/g dm (Table 5.4). Conversely, anthocyanin content increased as the severity of moisture stress increased, but no consistent trend was observed with CV17I and CV18-1A. The application of 30 kg P ha^{-1} application under 15 kPa irrigation level substantially increased the flavonoid and TSS content to 2.29 mg g^{-1} dm and 20.02^{O}Brix (Table 5.5), respectively, while the 30 kg P ha^{-1} irrigated at 50 kPa significantly increased the anthocyanin by about 55%. Interestingly, the 60 kg P ha^{-1} application with irrigation at 15 kPa (well-watered regime) resulted in a 10% increase in P content (Table 5.5). In contrast, the same P rate under a moderate moisture regime (50 kPa) resulted in increased Fe content. However, 90 kg P ha^{-1} with moderate moisture stress irrigated at 50 kPa increased the Zn content.

Table 5.3: Interaction effect of genotype and P levels regimes on secondary metabolites, protein and mineral composition cowpea grain

TMF	**Flv-2**	**TSS**	**ACN**	**%P**	**Fe**	**Zn**
G1xP0	2.13^{bcd}	19.66^{a}	0.724^{ab}	0.518^{c}	91.08^{b}	37.83^{h}
G1xP1	2.05^{de}	18.18^{ab}	0.718^{abc}	0.530^{b}	56.33^{j}	37.75^{h}
G1xP2	1.75^{gh}	19.90^{a}	0.533^{cdef}	0.533^{ab}	344.50^{a}	36.67^{i}
G1xP3	1.94^{ef}	17.37^{c}	0.622^{abcd}	0.520^{c}	60.17^{i}	36.17^{i}
G2xP0	2.09^{cd}	18.80^{ab}	0.427^{efg}	0.480^{g}	74.50^{de}	43.00^{e}
G2xP1	2.12^{cd}	18.94^{ab}	0.759^{ab}	0.497^{de}	76.42^{d}	42.00^{f}
G2xP2	2.19^{bc}	19.63^{a}	0.692^{abc}	0.495^{ef}	74.17^{e}	43.42^{de}
G2xP3	2.39^{a}	19.23^{a}	0.577^{bcde}	0.470^{h}	74.00^{e}	41.25^{g}
G3xP0	2.10^{cd}	18.96^{ab}	0.380^{fg}	0.497^{de}	71.75^{f}	43.33^{de}
G3xP1	2.16^{bcd}	18.38^{ab}	0.794^{a}	0.488^{f}	71.17^{f}	43.75^{d}
G3xP2	2.17^{bcd}	19.90^{a}	0.783^{a}	0.503^{d}	71.08^{f}	42.83^{e}
G3xP3	2.19^{bc}	18.70^{ab}	0.479^{defg}	0.473^{gh}	67.92^{g}	41.33^{fg}
G4xP0	2.26^{b}	18.60^{ab}	0.334^{g}	0.538^{a}	62.17^{h}	49.75^{a}
G4xP1	1.84^{fg}	19.92^{a}	0.373^{fg}	0.518^{c}	61.33^{hi}	44.50^{c}
G4xP2	2.11^{cd}	18.21^{ab}	0.380^{fg}	0.538^{a}	61.42^{hi}	48.68^{b}
G4xP3	1.68^{h}	19.34^{a}	0.419^{efg}	0.522^{c}	88.17^{c}	49.42^{a}
! CV (%)	3.73	8.32	20.32	0.64	1.08	0.75

TMF = Treatment factors, TSS= Total Soluble Solids (^{O}Brix), Pro=Protein (%), Flv-2= Flavonoid (mg/g dm), ACN = Anthocyanin (mg/g dm), %P = Phosphorus(%), Zn = Zinc (mg/kg), Fe = Iron (mg/kg), GxP implies treatment interaction of genotype (G) and phosphorus (P) levels, G1 = CV17I, G2 = CV17F, G3 = CV17B, G4 = CV18-1A, P0 = 0 kg P ha^{-1}, P1 = 30 kg P ha^{-1}, P2 = 60 kg P ha^{-1}, P3 =90 kg P ha^{-1} and ! CV = Coefficient of variation.

Table 5.4: Genotypes and moisture regimes interaction effect on secondary metabolites, protein and mineral composition cowpea grain

TMF	**Flv-2**	**ACN**	**%P**	**Fe**	**Zn**
G1xM1	2.19^{cd}	0.534^{c}	0.544^{a}	60.12^{h}	36.38^{h}
G1xM2	1.85^{f}	0.632^{abc}	0.525^{c}	273.69^{a}	37.94^{f}
G1xM3	1.86^{f}	0.781^{a}	0.508^{d}	80.25^{b}	37.00^{g}
G2xM1	2.14^{de}	0.598^{bc}	0.488^{ef}	80.06^{b}	41.88^{e}
G2xM2	2.19^{cd}	0.735^{ab}	0.478^{g}	72.75^{de}	43.19^{d}
G2xM3	2.26^{bc}	0.508^{cd}	0.491^{e}	71.50^{e}	42.19^{e}
G3xM1	2.06^{e}	0.372^{de}	0.510^{d}	67.69^{f}	42.88^{d}
G3xM2	2.34^{ab}	0.714^{ab}	0.476^{g}	74.62^{c}	43.81^{c}
G3xM3	2.07^{e}	0.742^{ab}	0.485^{f}	69.13^{f}	41.75^{e}
G4xM1	2.41^{a}	0.221^{e}	0.544^{a}	61.81^{g}	47.69^{b}
G4xM2	1.89^{f}	0.539^{c}	0.535^{b}	68.88^{f}	49.00^{a}
G4xM3	1.61^{g}	0.370^{de}	0.509^{d}	74.13^{cd}	47.56^{b}
! CV (%)	3.73	20.32	0.64	1.08	0.75

TMF = Treatment factors, Flv-2= Flavonoid (mg/g dm), ACN = Anthocyanin (mg/g dm), %P = Phosphorus (%), Zn = Zinc (mg/kg), Fe = Iron (mg/kg), GxM implies treatment interaction of genotype (G) and moisture (M) regime, G1 = CV17I, G2 = CV17F, G3 = CV17B, G4 = CV18-1A, M1 = 15 kPa, M2 = 50 kPa, M3 = 75 kPa and ! CV = Coefficient of variation.

Table 5.5: Interaction effect of P levels and moisture regimes on secondary metabolites, protein and mineral composition cowpea grain

TMF	Flv-2	TSS	ACN	%P	Fe	Zn
P0xM1	2.22^{a}	19.94^{a}	0.458^{cde}	0.528^{a}	71.25^{e}	43.19^{bc}
P0xM2	2.21^{a}	17.58^{bc}	0.502^{cde}	0.498^{e}	69.50^{f}	43.75^{a}
P0xM3	2.01^{b}	19.49^{ab}	0.438^{de}	0.500^{e}	83.87^{b}	43.50^{ab}
P1xM1	2.29^{a}	20.02^{a}	0.389^{e}	0.516^{b}	67.50^{gh}	42.13^{de}
P1xM2	1.85^{c}	16.71^{c}	0.731^{ab}	0.506^{cd}	68.25^{fg}	42.88^{c}
P1xM3	1.97^{b}	19.84^{a}	0.863^{a}	0.503^{de}	63.19^{i}	41.00^{f}
P2xM1	1.99^{b}	18.93^{ab}	0.465^{cde}	0.531^{a}	66.38^{h}	42.88^{c}
P2xM2	2.21^{a}	19.56^{ab}	0.790^{a}	0.511^{bc}	278.44^{a}	43.50^{ab}
P2xM3	1.97^{b}	19.75^{a}	0.536^{cde}	0.510^{c}	68.56^{fg}	42.31^{d}
P3xM1	2.29^{a}	16.47^{c}	0.413^{de}	0.510^{c}	64.56^{i}	40.63^{f}
P3xM2	2.00^{b}	19.76^{a}	0.596^{bc}	0.499^{e}	73.75^{d}	43.81^{a}
P3xM3	1.85^{c}	19.76^{a}	0.564^{cd}	0.480^{f}	79.38^{c}	41.69^{e}
! CV (%)	3.73	8.32	20.32	0.64	1.08	0.75

TMF = Treatment factors, TSS= Total Soluble Solids (^{O}Brix), Flv-2= Flavonoid (mg/g dm), ACN = Anthocyanin (mg/g dm), %P = Phosphorus (%), Zn = Zinc (mg/kg), Fe = Iron (mg/kg), GxM implies treatment interaction of phosphorus (P) levels and moisture (M) regime, P0 = 0 kg P ha^{-1}, P1 = 30 kg P ha^{-1}, P2 = 60 kg P ha^{-1}, P3 =90 kg P ha^{-1}, M1 = 15 kPa, M2 = 50 kPa, M3 = 75 kPa and ! CV = Coefficient of variation.

5.5.4 Second-order interaction effect on secondary metabolite and mineral composition of cowpea grains

Table 5.6 below highlights the second-order interaction effect on cowpea grain secondary metabolites and mineral contents. The CV18-1A genotype fertilized at 30 kg P ha^{-1} under well-watered (15 kPa) conditions significantly enhanced flavonoid content to 2.768 mg/g dm. However, when CV17B genotype fertilized at 30 kg P ha^{-1} under adequate soil moisture regime (15 kPa) increased the TSS content contrary to CV17I genotype fertilized at 90 kg P ha^{-1} also with adequate soil moisture regime where 38% reduction on the TSS content of the grain (Table 5.6). Interestingly the robust response of CV17B genotype fertilized at 30 kg P ha^{-1} at 75 kPa irrigation produced 96.9% higher grain anthocyanin content than CV18-1A genotype without any P addition at the same irrigation level. Furthermore, CV17I genotype fertilized at 60 kg P ha^{-1} under moderate water stress level (50 kPa) gave the highest Fe content of 902.5 mg/kg while grains from CV18-1A genotype fertilized at 90 kg P ha^{-1} application under severe water stress regime cause significant increase Zn content by 33.96% remarkably.

Table 5.6: The genotype x P levels x soil moisture regimes interaction effect on secondary metabolites and mineral composition cowpea grains

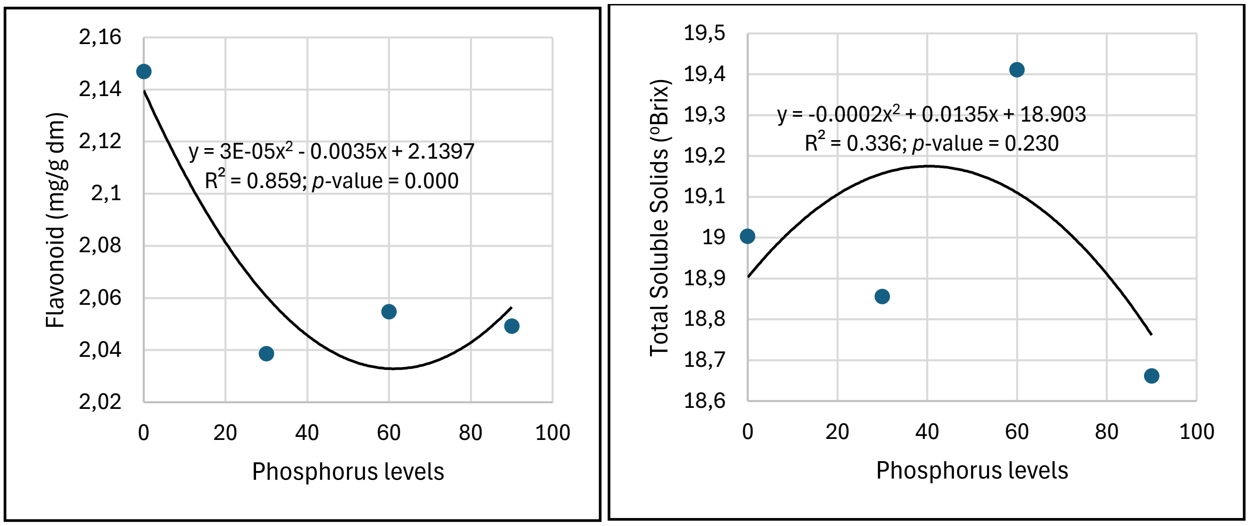
TMF	Flv-2	TSS	ACN	%P	Fe	Zn
G1xP0xM1	2.46^{bcde}	20.17^{ab}	0.646^{d-l}	0.525^{fgh}	64.50^{r-w}	35.00^{t}
G1xP0xM2	2.10^{i-o}	19.20^{abc}	0.746^{c-h}	0.530^{efg}	67.25^{o-s}	37.00^{pq}
G1xP0xM3	1.84^{pqr}	19.60^{abc}	0.779^{b-h}	0.500^{klm}	141.50^{b}	41.50^{lmn}
G1xP1xM1	2.42^{c-g}	20.07^{abc}	0.807^{b-g}	0.540^{cde}	53.25^{ab}	37.25^{p}
G1xP1xM2	1.59^{rst}	14.90^{bcd}	0.351^{j-q}	0.520^{ghi}	62.75^{t-x}	40.25^{no}
G1xP1xM3	2.15^{h-n}	19.57^{abc}	0.996^{abcd}	0.530^{efg}	53.00^{ab}	35.75^{qrst}
G1xP2xM1	1.78^{qr}	20.03^{abc}	0.406^{h-q}	0.570^{b}	56.50^{za}	36.75^{pqr}
G1xP2xM2	1.74^{qrs}	19.77^{abc}	0.607^{e-l}	0.530^{efg}	902.50^{a}	37.75^{p}
G1xP2xM3	1.72^{qrst}	19.90^{abc}	0.585^{e-l}	0.500^{klm}	74.50^{ghij}	35.50^{rst}
G1xP3xM1	2.09^{i-p}	12.57^{d}	0.278^{k-q}	0.540^{cde}	66.25^{p-u}	36.50^{pqrs}
G1xP3xM2	1.98^{l-q}	19.63^{abc}	0.824^{b-f}	0.520^{ghi}	62.25^{u-x}	36.75^{pqr}
G1xP3xM3	1.73^{qrs}	19.90^{abc}	0.763^{b-h}	0.500^{klm}	52.00^{b}	35.25st
G2xP0xM1	1.94^{m-q}	19.77^{abc}	0.618^{d-l}	0.480^{opq}	87.25^{e}	41.75^{klm}
G2xP0xM2	2.17^{f-m}	17.03^{abcd}	0.050^{q}	0.470^{qrs}	73.00^{hijk}	45.25^{gh}
G2xP0xM3	2.18^{f-m}	19.60^{abc}	0.612^{e-l}	0.490^{mno}	63.25^{s-x}	42.00^{klm}
G2xP1xM1	2.43^{bcdef}	19.43^{abc}	0.445^{f-p}	0.490^{mno}	91.50^{d}	42.00^{klm}
G2xP1xM2	1.86^{opq}	17.43^{abcd}	1.063^{abc}	0.490^{mno}	67.75^{n-r}	41.25^{mno}
G2xP1xM3	2.06^{j-p}	19.97^{abc}	0.768^{b-h}	0.510^{ijk}	70.00^{k-p}	42.75^{ijkl}
G2xP2xM1	1.93^{m-q}	20.20^{a}	0.651^{d-k}	0.495^{lmn}	74.75^{ghij}	43.50^{ij}
G2xP2xM2	2.05^{k-p}	19.10^{abc}	1.136^{ab}	0.490^{mno}	77.75^{g}	42.75^{ijkl}
G2xP2xM3	2.60^{abc}	19.60^{abc}	0.289^{k-q}	0.500^{klm}	70.00^{k-p}	44.00^{hi}
G2xP3xM1	2.27^{d-k}	17.97^{abc}	0.679^{d-j}	0.485^{nop}	66.75^{opqrst}	40.25^{no}

G2xP3xM2	2.68^{ab}	19.77^{abc}	0.690^{c-j}	0.460^{st}	72.50^{ijklm}	43.50^{ij}
G2xP3xM3	2.22^{d-l}	19.97^{abc}	0.362^{i-q}	0.465^{rs}	82.75^{f}	40.00^{o}
G3xP0xM1	2.31^{d-j}	20.10^{abc}	0.161^{n-q}	0.520^{ghi}	68.50^{m-r}	45.75^{fg}
G3xP0xM2	2.16^{g-m}	17.23^{abcd}	0.657^{d-k}	0.460^{st}	76.00^{ghi}	42.25^{jklm}
G3xP0xM3	1.84^{pqr}	19.53^{abc}	0.323^{j-q}	0.510^{ijk}	70.75^{j-o}	42.00^{klm}
G3xP1xM1	1.59^{rst}	20.37^{a}	0.134^{opq}	0.500^{klm}	67.75^{n-r}	42.25^{jklm}
G3xP1xM2	2.47^{bcd}	14.87^{cd}	0.996^{abcd}	0.475^{pqr}	77.00^{gh}	46.00^{fg}
G3xP1xM3	2.42^{cdefg}	19.90^{abc}	1.252^{a}	0.490^{mno}	68.75^{l-q}	43.00^{ijk}
G3xP2xM1	1.89^{nopq}	20.17^{ab}	0.679^{d-j}	0.520^{ghi}	71.75^{j-n}	43.50^{ij}
G3xP2xM2	2.57^{abc}	19.70^{abc}	0.935^{a-e}	0.500^{klm}	72.75^{ijkl}	45.00^{gh}
G3xP2xM3	2.06^{j-p}	19.83^{abc}	0.735^{c-i}	0.490^{mno}	68.75^{l-q}	40.00^{o}
G3xP3xM1	2.46^{bcde}	17.00^{abcd}	0.512^{f-o}	0.500^{klm}	62.75^{t-x}	40.00^{o}
G3xP3xM2	2.15^{h-m}	19.77^{abc}	0.267^{l-q}	0.470^{qrs}	72.75^{ijkl}	42.00^{klm}
G3xP3xM3	1.98^{l-q}	19.33^{abc}	0.657^{d-k}	0.450^{t}	68.25^{n-r}	42.00^{klm}
G4xP0xM1	2.15^{h-m}	19.73^{abc}	0.406^{h-q}	0.585^{a}	64.75^{q-w}	50.25^{b}
G4xP0xM2	2.42^{c-g}	16.83^{abcd}	0.557^{e-m}	0.530^{efg}	61.75^{vwx}	50.50^{b}
G4xP0xM3	2.20^{e-l}	19.23^{abc}	0.039^{q}	0.500^{klm}	60.00^{xyz}	48.50^{cd}
G4xP1xM1	2.77^{a}	20.20^{a}	0.173^{n-q}	0.535^{def}	57.50^{yz}	47.00^{ef}
G4xP1xM2	1.49^{stu}	19.63^{abc}	0.512^{f-o}	0.540^{cde}	65.50^{q-v}	44.00^{hi}
G4xP1xM3	1.25^{uv}	19.93^{abc}	0.434^{g-p}	0.480^{opq}	61.00^{wxy}	42.50^{jklm}
G4xP2xM1	2.34^{c-i}	15.30^{abcd}	0.123^{pq}	0.540^{cde}	62.50^{u-x}	47.75^{de}
G4xP2xM2	2.46^{bcde}	19.67^{abc}	0.484^{f-p}	0.525^{fgh}	60.75^{wxy}	48.50^{cd}
G4xP2xM3	1.51^{st}	19.67^{abc}	0.534^{f-n}	0.550^{c}	61.00^{wxy}	49.75^{bc}
G4xP3xM1	2.36^{c-h}	18.33^{abc}	0.184^{m-q}	0.515^{hij}	62.50^{u-x}	45.75^{fg}
G4xP3xM2	1.19^{v}	19.87^{abc}	0.601^{e-l}	0.545^{cd}	87.50^{de}	53.00^{a}
G4xP3xM3	1.47^{tu}	19.83^{abc}	0.473^{f-p}	0.505^{jkl}	114.50^{c}	49.50^{bc}
! CV (%)	3.73	8.32	20.32	0.64	1.08	0.75

TMF = Treatment factors, TSS= Total Soluble Solids (^{O}Brix), Pro=Protein (%), Flv-2= Flavonoid (mg/g dm), ACN = Anthocyanin (mg/g dm), %P = Phosphorus (%), Zn = Zinc (mg/kg), Fe = Iron (mg/kg), GxPxM implies treatment interaction genotype (G), phosphorus (P) levels and moisture (M) regime, G1 = CV17I, G2 = CV17F, G3 = CV17B, G4 = CV18-1A, P0 = 0 kg P ha^{-1}, P1 = 30 kg P ha^{-1}, P2 = 60 kg P ha^{-1}, P3 =90 kg P ha^{-1}, M1 = 15 kPa, M2 = 50 kPa, M3 = 75 kPa and ! CV = Coefficient of variation.

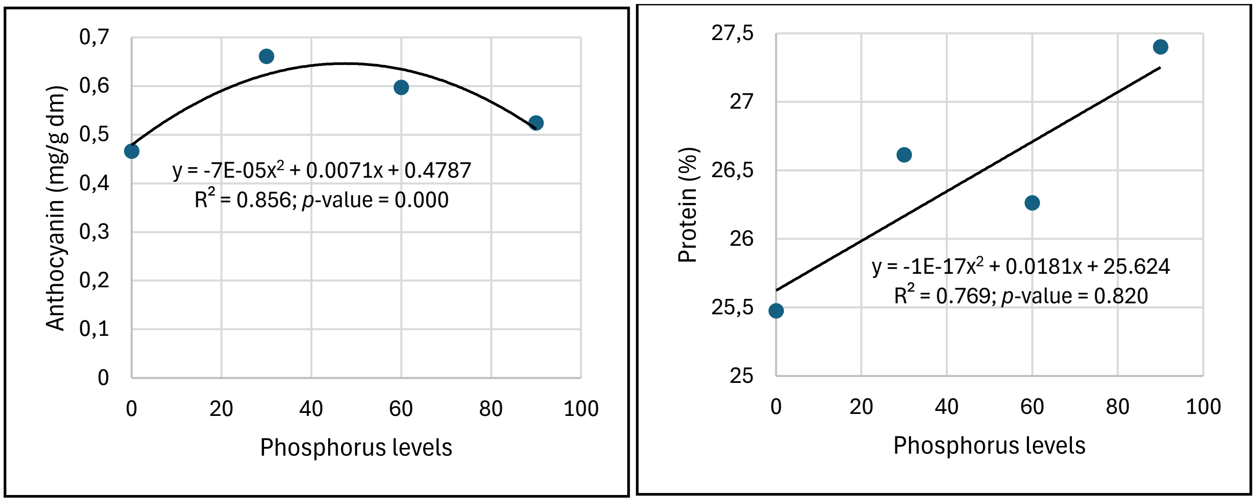
5.5.5 Regression, correlation, PCA, and cluster analysis

Figure 5.2 below outlines the quadratic polynomial regression responses of the various measured cowpea grain parameters (i.e., secondary metabolites, protein content, and mineral composition) to varying P fertilization levels. Except for the protein content that showed a strong linear and positive but non-significant response (R^2 = 0.789) to P fertilizer application, the responses of flavonoid, anthocyanin, and mineral P, Zn, and Fe concentrations exhibited strongly high R-square that ranged from 0.301 to 0.859. Unexpectedly the response for the total soluble solids was moderate R^2 (0.336) and non-significant.



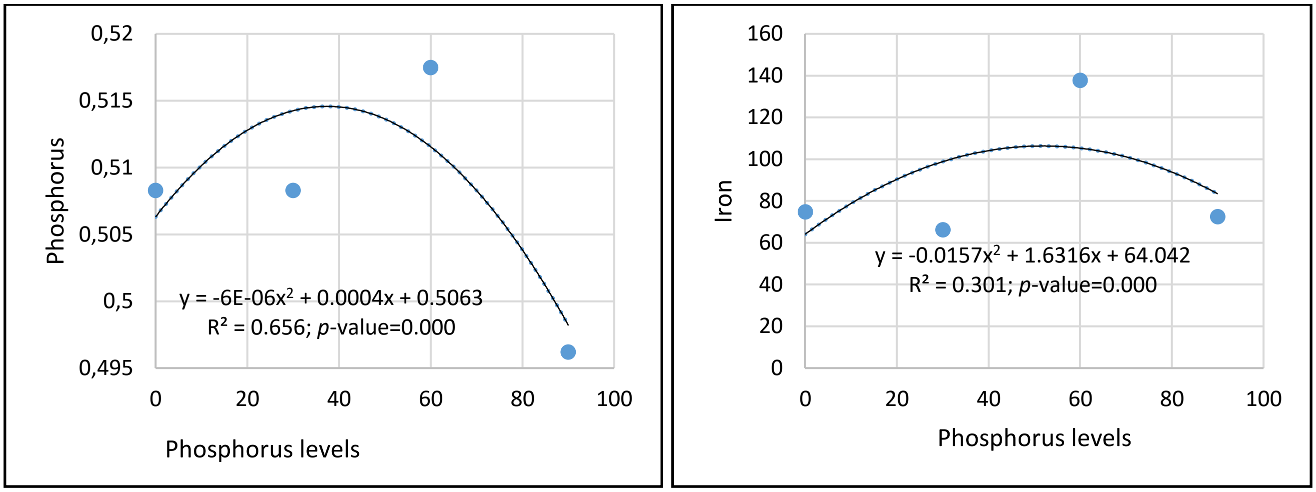
A. Phosphorus levels versus flavonoid

B. Phosphorus versus total soluble solids



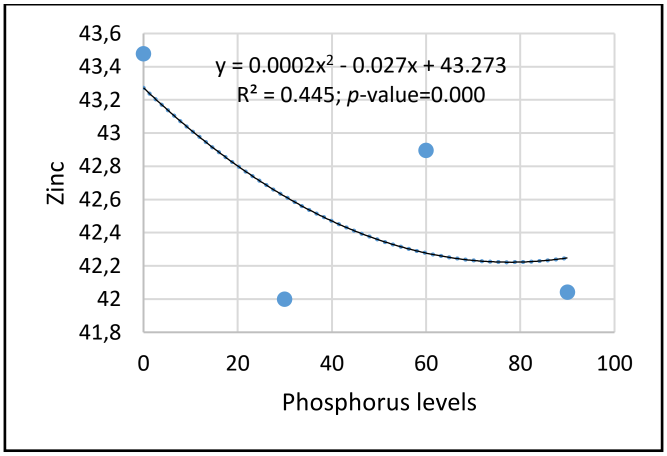
C. Phosphorus versus anthocyanin

D. Phosphorus versus protein



E. Phosphorus versus phosphorus content

F. Phosphorus versus Iron



G. Phosphorus versus Zinc

Figure 5.2: Quadratic polynomial of phosphorus (P) fertilizer application rate on flavonoid (A), total soluble solids (B), anthocyanin (C), protein (D), phosphorus content (E), Iron (F) and Zinc (G).

The correlation matrix displayed in Figure 5.3 reveals a strongly significant though negative correlation between protein and TSS. Similarly, a significant and negative correlation between Zn and anthocyanin content was observed, while P and anthocyanin contents exhibited a negative and non-significant correlation. The association between Fe and either TSS, anthocyanin, and P content and TSS and flavonoid, revealed a positive correlation. The component loading of the seven principals for secondary metabolites, protein, and mineral content of cowpea grain assessed revealed 21.37% as the highest variability recorded with the most important traits' loads on PC1

(Table 5.7). While the flavonoid content had a substantially low and negative association with PC1, PC2, and PC3, iron similarly recorded a negative association with PC5, PC6, and PC7 (Table 5.7). Interestingly, phosphorus content positively correlated with the PC levels except for PC1, which has a negative correlation. The Eigenvalue of the PC decreases as PCs increase, with a range of 0.10 to 1.49 (Table 5.7).

A graphical biplot display presented in Figure 5.4 outlines the PCA of all the assessed secondary metabolites, protein, and mineral composition of cowpea grain. The biplot provides a data structure, relationships between parameters, and clusters of similar observations. It reveals that the anthocyanin and TSS contents are dominantly associated with C1, indicating major factors in the distribution of the relationships in the data structure (Figure 5.4). In contrast, protein and flavonoids majorly have a negative effect on C2, with a high association with C1. Remarkably, the Zn content primarily contributes to the negative composition of C1 and is largely independent of the other variables. Interestingly, the percent P content contributed a relatively moderate effect to C2, with a limited overall expression on the PCA display (Figure 5.4).

The hierarchical cluster in Figure 5.5 presents a strong similarity scale that ranges between 0.950 and 1.000. The association between CV17B genotype and 30 kg P ha^{-1} (G3xP1) and 60 kg P ha^{-1} under severe moisture regime (P2xM2) depicted a very strong similarity scale, which is similar to CV17I genotype under well water regime (G1xM1) and CV18-1A with 30 kg P ha^{-1} application under moderate water regime (G4xP1xM2) despite being very far from the other group. Inversely, observations of 90 kg P ha^{-1} under a well-watered regime (P3xM1) and CV17I with the application of 30 kg P ha^{-1} under a moderately watered regime (G1xP1xM2) presented a very strong association with a very strong similarity level.

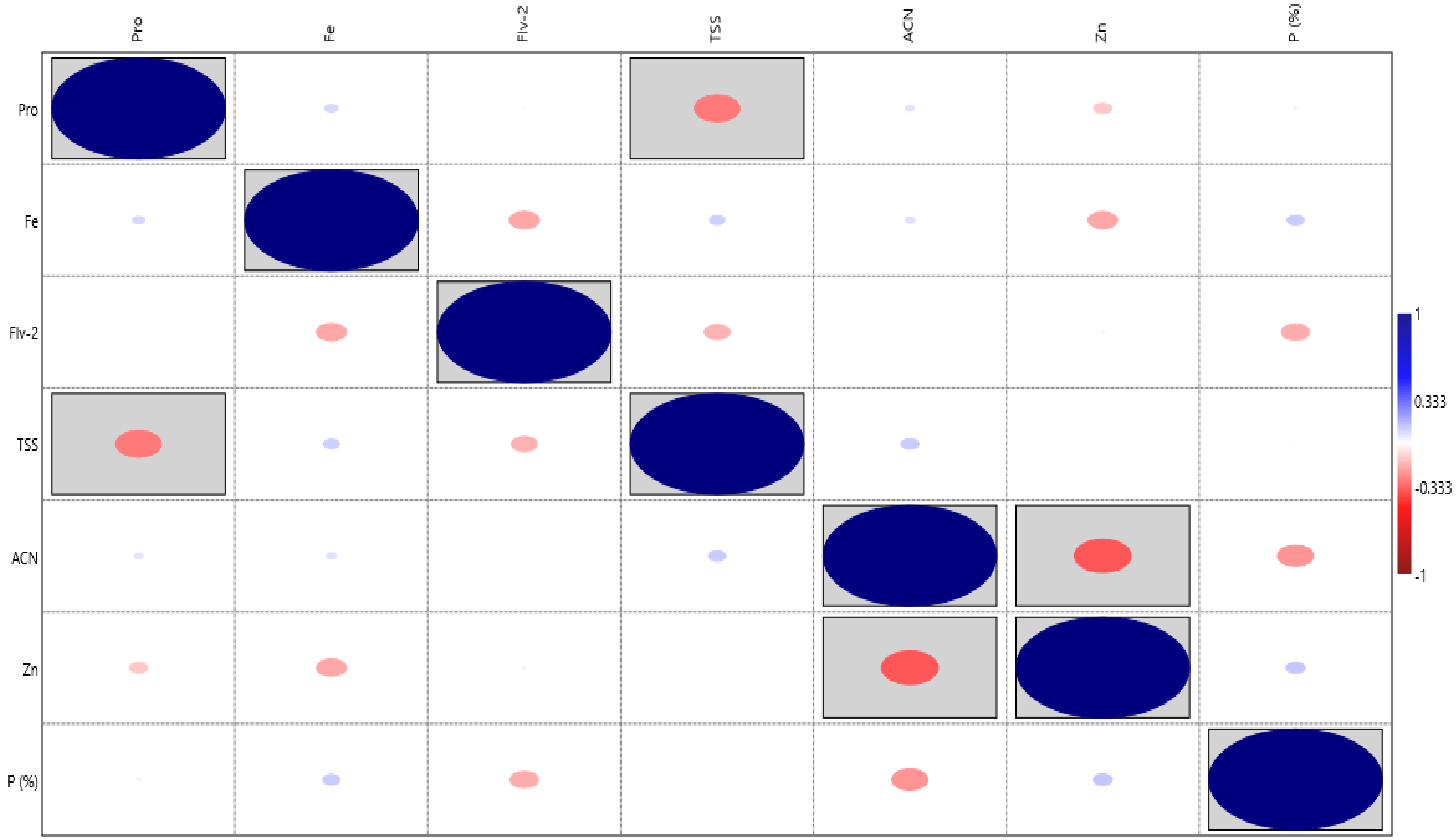


Figure 5.3: Circles correlation matrix plot within the secondary metabolites, protein, and mineral contents of cowpea grain.

Table 5.7: Loadings of the measured traits onto seven principal components among secondary metabolites, protein and mineral composition of cowpea

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Protein (%)	0.1614	-0.2784	0.6343	-0.2358	-0.2321	0.5482	-0.2899
Iron	0.2883	0.4275	0.344	0.6332	-0.3899	-0.0267	0.2492
Flavonoid	-0.0479	-0.5789	-0.1953	0.6885	0.2246	0.3128	-0.0462
TSS	0.1225	0.5095	-0.482	0.0335	-0.0367	0.5496	-0.4342
Anthocyanin	0.6219	-0.0637	-0.1581	-0.2308	0.2489	0.2993	0.6159
Zinc	-0.6198	0.0141	-0.1254	-0.0889	-0.3917	0.4069	0.5224
Phosphorus	-0.3202	0.3751	0.4102	0.0846	0.7258	0.2091	0.1042
Eigenvalue	1.49559	1.3707	1.26158	0.804075	0.756595	0.699773	0.611685
Variance %	21.366	19.581	18.023	11.487	10.809	9.9968	8.7384

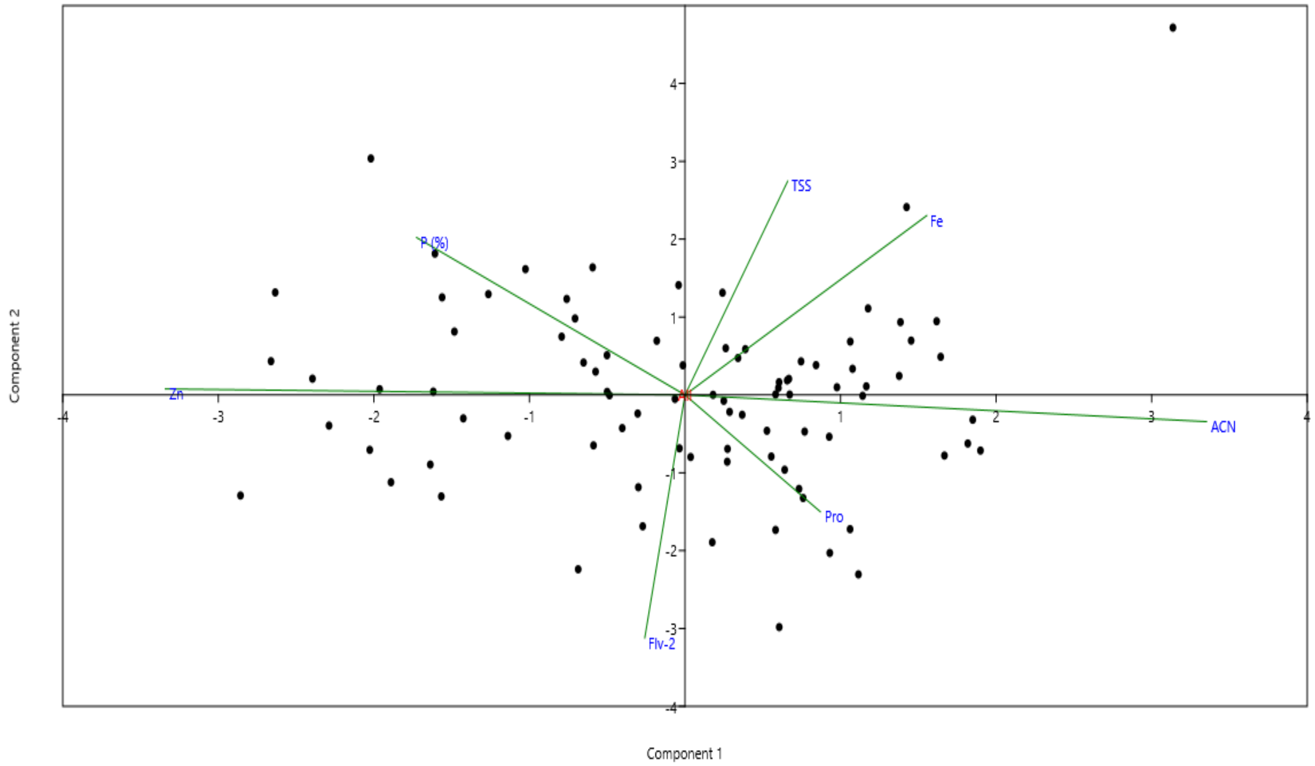


Figure 5.4: Biplot graphical display of the measured secondary metabolites, protein and mineral composition of cowpea grain.

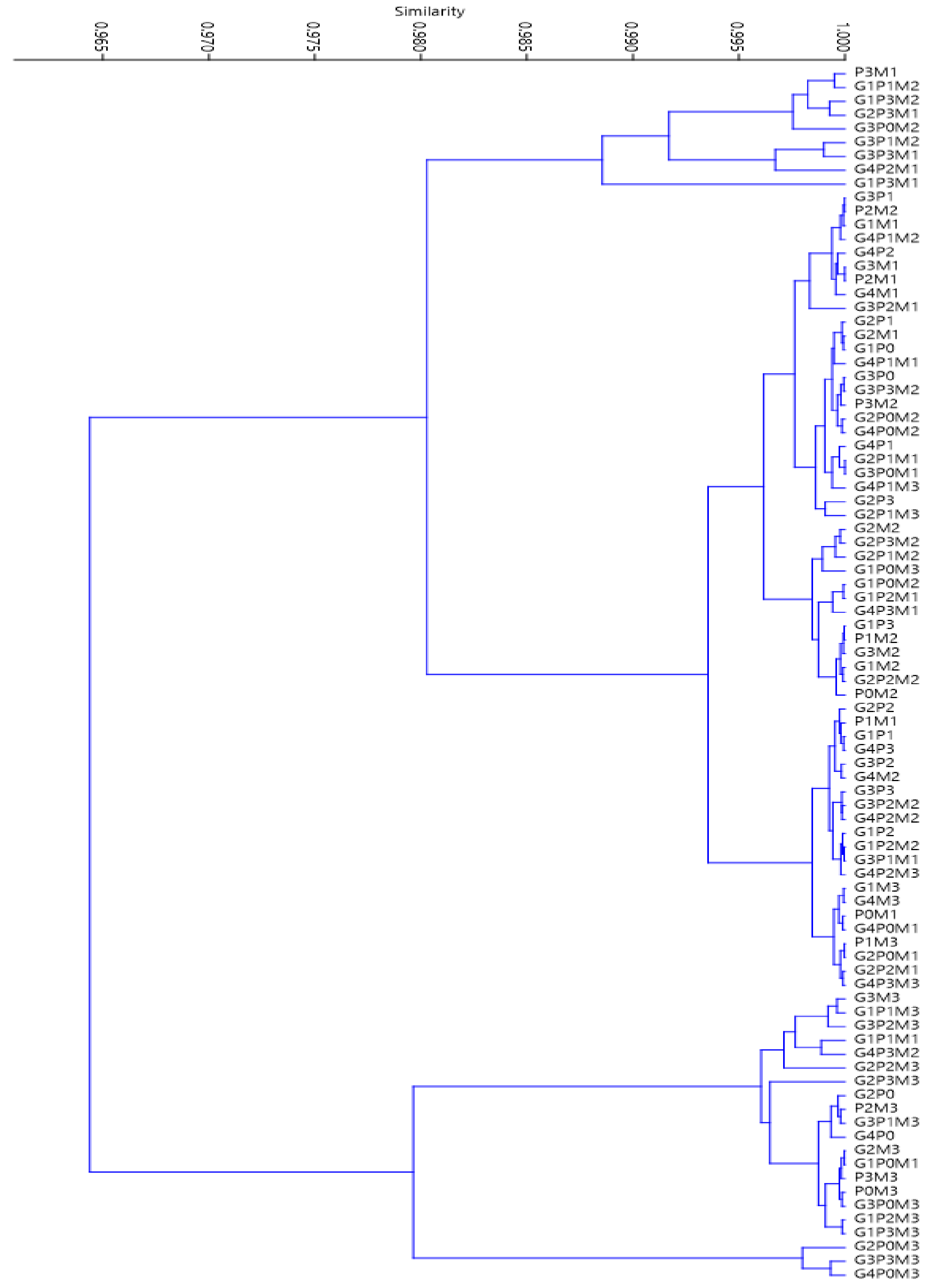


Figure 5.5: Cluster analysis of cowpea genotypes, P Levels, and moisture regimes interaction effect using group averages and Euclidean distance methods.

5.6 Discussion

5.6.1 Main treatments and their interaction on cowpea grain secondary metabolites

Flavonoid

Flavonoids play a crucial part in protecting plants against ultraviolet radiation, increasing their resilience to drought and cold temperatures, and increasing their ability to resist plant pathogens, thereby aiding plants in coping with both biotic and abiotic stress (Baozhu *et al.,* 2022; Bai *et al.,* 2020). Kozlowska and Szostak-Wegierek (2014) reported that flavonoid content in plants has a critical role in preventing cardiovascular diseases. The significantly higher flavonoid content where P was applied observed in the present study reveals that the level of inherent soil P availability affects cowpea secondary metabolomics by limiting flavonoid synthesis. This corroborates the earlier finding of Tewari *et al.* (2021), who reported that soil P deficiency can increase flavonoid content by enhancing antioxidant activity to protect against oxidative stress. Thus, the present study suggests that flavonoid production in cowpeas may not follow a uniform trend following an increase in the P fertilizer rate. The genotypic differences in flavonoid content indicate that the CV17F genotype produces higher flavonoid content than the CV17I and CV18-1A genotypes suggesting that genomic variation significantly influences the direct biosynthesis of secondary metabolites (Winkel-Shirley, 2001).

The observed reduction in flavonoid content by approximately 10.96% by moisture stress is possibly attributed to the redirection of energy toward self-defense pathways (Park *et al.,* 2023; Cetinkaya *et al.,* 2017). Additionally, the results elucidate the significant enhancement in flavonoid content of CV18-1A genotype fertilized at 30 kg P ha^{-1} under a well-watered regime, highlighting the optimal flavonoid production under the conditions. A review by Pant *et al.* (2021) on the influence of soil moisture conditions on secondary metabolites in medicinal plants highlights the interaction effect on secondary metabolite content in plants supporting the findings of the present study on the three-way interaction effecting the flavonoid content. Moreover, Baozhu *et al.* (2022) outlined that water deficit conditions induced flavonoid biosynthesis, consequently promoting drought acclimation. Other studies have shown that flavonoids regulate salinity and drought responses by removing reactive oxygen species (ROS) while inhibiting the activities of ROS-producing enzymes thus promoting the activities of antioxidant enzymes and repairing damage caused by ultraviolet radiation (Wang *et al.,* 2020; Li *et al.,* 2019).

Total Soluble Solids

A significant increase in TSS content observed under severe water stress as opposed to moderate water stress (15 kPa) aligns with the findings by Afzal *et al.* (2021) that water stress induces the accumulation of soluble sugars and osmolytes as part of plant response to water stress. This underscores the role of soluble sugars like glucose, fructose, and sucrose to act as osmoprotectants, which assist plants in maintaining cell turgor and protect cellular structures under moisture stress. An increase in TSS under water deficit conditions was observed by Ma *et al.* (2022) in fruits, which has a substantial implication on taste and nutritional quality as a strategy for inducing maturity by accumulating more sugars. The present study suggests that similar mechanisms have interplay in cowpeas, where water stress leads to an upregulation of pathways involved in sugar biosynthesis resulting in higher TSS levels. Interestingly, the PxM interaction significantly affected the TSS content leading to the high TSS in 30 kg P ha^{-1} fertilization irrigated at 15 kPa conditions. According to the findings of Wu *et al.* (2021) the application of P fertilizer increased TSS concentrations by improving sucrose metabolism.

The significant increase in TSS recorded with CV17B genotype fertilized at 30 kg P ha^{-1} under well-watered conditions (15 kPa) is comparable to that of the CV17I genotype fertilized at 90 kg P ha^{-1} under similar moisture conditions underlining the role of gene expression despite the low P application rate with CV17B highlighting the superior traits of improving TSS content. This result is consistent with the findings in wheat reported by Saini *et al.* (2024) and Yan *et al.* (2010). Additionally, the accumulation of TSS in cowpea grain may be strongly associated with the expression of enzymes involved in sucrose metabolism. Ma *et al.* (2022) reported that cell wall and invertase expression levels, vacuolar and invertase, and synthase under drought stress decrease in the maize ovary. Plant cells recognize and receive stress through signal sensors under water stress conditions and convert the extracellular signals to intracellular signals for transduction (Yang *et al.,* 2021). During this process, the second messenger generated when plants respond to soil moisture stress plays a significant role in signal transduction when plants experience stress. The initial signals caused by stress are converted into signals related to mechanical, osmotic, and oxidative stress (Gong *et al.,* 2020).

Anthocyanin content

Anthocyanins are part of a significant plant secondary metabolites under the flavonoid family, classified as water-soluble natural pigments of plants. The study's finding reveals a comparable genotypic effect amongst the tested genotype anthocyanin content, with CV17I, CV17F, and

CV17B genotypes showing a much higher accumulation of anthocyanin content than the CV18-1A. This finding suggests a strong metabolic pathway variation in different genotypes influences the anthocyanin synthesis in cowpeas. The observed variation among genotypes is consistent with the earlier findings by Li and Ahammed (2023), who reported a significant role of anthocyanins in stress tolerance through manipulating regulatory genes for potential use in plant stress resistance against abiotic stresses. Interestingly Horbowicz *et al.* (2008) reported the significant role of anthocyanin in both animals and humans to include the protection against a variety of diseases, particularly cardiovascular disease and cancer. Likewise, the observed influence of the variation in moisture regime on anthocyanin content reveals that water stress can induce the synthesis of anthocyanins as part of the plant's defense mechanism against oxidative stress caused by drought (Medina-Lozano *et al.*, 2024; Li and Ahammed, 2023). However, it can be argued that anthocyanins protect cells by scavenging reactive oxygen species and stabilizing cellular membranes under stress conditions (Naing and Kim, 2021). This justifies the significant increase in anthocyanin content under severe water stress with P application. Moreover, the significant three-way GxPxM interaction further points to the integrated agronomic strategy's significant role in enhancing cowpeas' anthocyanin content. The approximate two-fold (nearly 97%) increase in anthocyanin content in CV17B genotype irrigated at 15 kPa with 30 kg P ha^{-1} over the CV18-1A genotype irrigated under similar moisture conditions but without P underscores the critical role of P in intensifying stress leading to induced anthocyanins production.

5.6.2 Main treatments and their interaction on cowpea grain protein content

The present study underlined a significant differential effect of soil moisture on protein content, revealing that irrigation at 15 kPa resulted in 27.64% enhanced protein synthesis compared to water stress conditions that reduced protein content by up to 22.92% depending on the severity This is supported by the work of Wen *et al.* (2018), which revealed that soil moisture availability has a direct effect on protein synthesis, affecting the physiological processes of plants through N and P uptake. The intensification of moisture stress disrupts these processes, thereby reducing the availability of amino acids and the necessary precursors for protein synthesis. Similar trends have been reported in soybean and chickpeas, where moderate drought stress led to increased protein content Ghotbi-Ravandi *et al.* (2021), conceivably due to increased N concentration because of reduced biomass accumulation under stress conditions. In contrast, severe drought stress was associated with a significant reduction in protein content due to impaired P assimilation for plant metabolism. The significant PxG interaction effect on the protein content despite P activating metabolic pathways in plants for protein synthesis, as reported in numerous studies (Su *et al.* 2024; Prathap *et al.* 2023; Ishihara *et al.* 2015). The observed 57.14% increase in protein content of CV17I genotype fertilized at 90 kg P ha^{-1} under moderate water stress (50 kPa) compared to the CV17F genotype without P addition irrigated at 75 kPa agrees with cowpea studies by Dekhane *et al.* (2011) who reported that increasing P application with adequate irrigation can improve the grain protein content of cowpea.

5.6.3 Main treatments and their interaction with cowpea grain mineral composition

Phosphorus

Phosphorus is one of the essential minerals in food predominantly stored in the form of phytate in grains. The current findings revealed that P fertilization beyond 60 kg ha^{-1} caused a 4.16% reduction in P content in cowpea grain, suggesting a P threshold of 60 kg ha^{-1} beyond which antagonistic interaction is triggered protracted plant growth rather than improving grain content as reported by Mohammed *et al.* (2021). The significant GxPxM interaction effect on grain P content potentially indicates that the cowpea's ability to accumulate P in seeds is not solely dependent on P availability but also on moisture conditions and genotypic factor integration to promote PUE. Likewise, the CV17B genotype fertilized at 90 kg P ha^{-1} under severe water stress resulted in 23.1% lower P content compared to the CV18-1A genotype without P addition under a well-watered regime suggesting that water stress with high application of P does not improve the P content.

The reduced P content in the CV17B genotype suggests this genotype has less PUE in maintaining P uptake and translocation under moisture stress conditions except when excess P is applied contrary to the findings of Huang *et al.* (2011) reporting that genotypic differences increase PUE which is crucial when phosphorus supply is restricted for optimal growth, and genotypes that exhibit higher PUE under low phosphorus conditions are advantageous. However, the highest grain P content, despite the adequate soil available P with CV18-1A genotype, is possibly due to the inheritable vigorous genetic and physiological makeup of the plant under a well-watered regime. This finding aligns with similar findings reported by Jin *et al.* (2006) that drought stress limits P translocation to the seed of two soybean genotypes irrespective of P treatment application. The result is further corroborated by Iqbal *et al.* (2019), who emphasized that genotypic variation showed high P accumulation under moderate P levels. The observed reduction in grain P content under severe water stress also agrees with the finding of Saha *et al.* (2022) that the decreased nutrient absorption during drought is caused by a decrease in mineralization, nutrient movement, and mass flow in the soil, which impacts the speed at which roots can take up nutrients (Bárzana and Carvajal, 2020). According to Asiwe (2022), the nutritional value of cowpea grains depends on the availability of nutrients in the soil for the plant to absorb. This means that if the soil lacks sufficient nutrients, the plant may not take up enough, leading to a lower concentration of nutrients in the grains.

Iron

The significant variation in the measured Fe content of cowpea grain exhibited following P application, with the highest value obtained at 60 kg P ha^{-1} application, implies that enhanced Fe uptake and translocation to the seeds can be influenced by P application. Similarly, Tran *et al.* (2021) reported that though P fertilizer application increases wheat grain yield, it also reduces the bioavailability of grain Zn and Fe content due to low plant uptake efficiencies of the available P. This study's observed genotypic differences in Fe content agree with the literature. Lenna *et al.* (2023) reported that cowpea genotypic differences significantly influence the Fe content in seeds, similar to the present findings of CV18-1A displaying the highest (54.45 mg/kg) Fe concentration. The significant influence of the variation in soil moisture regime observed on Fe content under moderate moisture regime leads to the highest (122.48 mg/kg) Fe content. At the same time, the well-watered conditions resulted in a 44.95% reduction in Fe content. These findings align with a study by Islam and Sandhi (2023), which revealed that moderate watering has been found to enhance micronutrient accumulation, including Fe. Similarly, Shoormij *et al.* (2023) reported that grain yield decreased due to water stress, while the grain quality traits such as protein, Zn, and Fe contents increased remarkably. Additionally, a moderate stress regime might have triggered adaptive responses of the plans through increasing root and shoot nutrient transport and concentration of nutrients in seeds as a survival mechanism as described by (Ghadirnezhad *et al.,* 2023; Seleiman *et al.,* 2021; Lo *et al.,* 2017).

In contrast, a well-watered regime might have caused leaching of nutrient concentrations due to higher biomass accumulation without a corresponding increase in nutrient uptake. The observed highest iron content (902.5 mg/kg) in the CV17I genotype with 60 kg P ha^{-1} under moderate moisture stress highlights the significance of optimizing soil moisture and P application to achieve high iron content. For instance, Chtouki *et al.* (2022) reported that chickpea genotypes exhibited higher iron content under drought stress when P fertilizer was optimally supplied, indicating a synergistic effect of moderate stress and phosphorus on iron uptake. The reduction in iron content under severe water stress, particularly with higher P application, might be due to impaired nutrient transport and metabolism under extreme stress conditions. Severe moisture stress can lead to reduced root growth, impaired nutrient absorption, and altered translocation processes, resulting in lower nutrient accumulation in seeds (Saha *et al.,* 2022). The significant three-way GxPxM interaction effect on iron content of cowpea grains underscores the complexity of nutrient management. The finding that the CV17I genotype with 60 kg P ha^{-1} under moderate stress produced cowpea grains with the highest iron content suggests that optimal nutrient and water management are crucial for specific genotypes and environmental conditions. Velu *et al.* (2016) similarly reported that drought stress and genotypic differences in different wheat lines significantly altered the proportion of grain iron and zinc concentrations.

Zinc

Zinc is an essential mineral found in different food sources, including leguminous crops such as cowpeas. It plays vital roles in bodily functionality, including the immune system, wound healing, and cell growth (Chasapis *et al.,* 2020). The findings of the current study revealed a positive and significant effect of P fertilization on grain Zn content, contradicting previous findings by Mohammed *et al.* (2021) and Ova *et al.* (2015), who reported that grain Zn content for various cowpea genotypes evaluated had an inconsequential effect across different P fertilization levels. This was also shown through correlation analysis, which indicated that grain Zn content exhibited a non-significant negative relationship with a high P rate compared to moderate and low rates (Mohammed *et al.*, 2021).

The highest zinc concentration in grains from the CV18-1A genotype in the present study suggests a genomic differential effect in cowpea's ability to accumulate Zn in grains. Similar findings have been reported by Azeem *et al.* (2023) following the planting of different wheat genotypes. This is consistent with results from soil with a relatively high Zn (7.64 mg/kg), indicating that the crop could access zinc from soils with more than 1.80 mg Zn/kg (Khokhar *et al.* (2024). Likewise, the imposition of severe soil moisture stress (75 kPa) significantly affects Zn content, leading to reduced grain Zn. The observed reduction in Zn content under severe water stress conditions is consistent with the findings by Choukri *et al.* (2020) that drought stress has a direct effect on soil nutrient uptake for the plant, disturbing nutrient translocation and distribution, thus affecting plant growth and altering nutrient metabolism. This might be attributed to the reduction of root nutrient uptake, by decreasing root biomass and metabolic rate (Heckathorn *et al.*, 2013). The observed 33.96% increase in Zn content in the CV18-1A genotype fertilized at 90 kg P ha^{-1} under severe water stress (75 kPa) compared to the CV17I genotype without P application but irrigated at 15 kPa suggests that an integrated soil-water management strategy can significantly improve cowpea grain Zn content, particularly in poor fertility soil with limited water availability. Malhotra (2018) generally reported that P enhances root growth and nutrient uptake, but its effectiveness can vary depending on soil moisture level. Hence, this study reveals that a strategy integrating genotypic variation with improved soil moisture and P application can potentially enhance the Zn content.

5.6.4 Correlation matrix, Regression, PCA, and cluster analyses among all measured parameters

The observed negative correlation between protein and TSS aligns with the findings of Chen *et al.* (2023), reporting that increasing the protein content often corresponds with reduced sugar accumulation. Additionally, the metabolic allocation shifts between protein biosynthesis and carbohydrate storage Seydel *et al.* (2022). The positive correlations between Fe and secondary metabolites (i.e. TSS, anthocyanin, and P content) suggest that Fe significantly enhances these compounds. The principal component analysis results highlight the dominant role of anthocyanins and TSS in PC1, a common trend in metabolomics where sugars and phenolics are key contributors to variability in plant composition (Zhong *et al.*, 2022; Cosme *et al.*, 2020). The negative association of flavonoids with PC1, PC2, and PC3 suggests a distinct metabolic regulation pathway, potentially indicating trade-offs in secondary metabolite synthesis.

5.7 Conclusion

The findings from the current study suggest that the P and moisture applications significantly adjust cowpea secondary metabolites, protein, and mineral contents. These two factors (i.e. soil P and moisture levels) are critical and play significant role in modifying the metabolic pathways that permit cowpea genotypes to tolerate harsh stress conditions such as drought stress. Interestingly, the induced response flow is entirely correlated to the photosynthesis efficiency that aligns with the plant’s ability to uptake nutrients and minerals. The interactive effect of the moisture regime x phosphorus level on cowpea genotypes indicates an integration strategy to promote metabolomic pathways and mineral accumulation in cowpea grain. Moreover, the findings demonstrate that the CV17I genotype fertilized at 90 kg P ha^{-1} under moderate irrigation level (50 kPa) can significantly increase protein content by over 57% in cowpea grains, which distinctly highlights the importance of integrated P and moisture regime management to improve cowpea protein nutrition. Additionally, moisture stress could significantly reduce cowpea grain flavonoid content by up to 11%; hence, this remains an imperative factor in cowpea production, including for the P fertilization program.

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CHAPTER 6

General Summary, Conclusion, and Recommendations

6.1 Summary of main findings of the study

6.1.1 Results of pre-planting soil analysis

The result of pre-planting laboratory analysis of the sample of soil used for the study revealed a slight acidity with a pH (KCl) value of 5.94 and a sandy loam textural class. The available Bray P1 measured content of 16 mg kg^{-1} suggests P adequacy for cowpea production.

Greenhouse trial

- The results of the study revealed that genotypic variation exerted a significant ($p \leq 0.05$) effect in all measured phenological and yield-related parameters, while P fertilization had an inconsequential effect except for total biomass and WUE at flowering. Similarly, the variation in soil moisture regimes exerted a significant ($p \leq 0.05$) effect on all measured phenological and yield parameters except for the HSW.
- The CV17I genotype with early flowering and pod initiation observed at 49 and 52 days after planting produced a 64% higher number of pods per plant than any other genotypes. On the other hand, the CV18-1A represents a late maturing genotype that produced flower and pod after 63 and 67 days, respectively. Similarly, the CV17B genotype is a medium to late maturing genotype that possesses a significantly ($p \leq 0.05$) highest leaf length and stomatal conductance.
- Irrigation level imposed at 15 kPa significantly induced early flowering and pod formation, while severe moisture stress extremely delayed flowering and pod formation up to 57 and 60 days after planting, respectively. Interestingly, most measured parameters (i.e., number of pods per plant, pod length, number of seeds per pod, number of cavities per pod, and seed weight per plant) were significantly ($p \leq 0.05$) improved by adequate irrigation regime (i.e., 15 kPa), the total biological yield and WUE at harvesting.
- The results of the study also revealed a significant ($p \leq 0.05$) GxM interaction effect on the mean number of trifoliate leaves and stomatal conductance, while the GxP interaction significantly ($p \leq 0.05$) affected cowpea leaf length. The CV18-1A genotype

produced the tallest plant and the highest number of leaves per plant, chlorophyll content, pod length, number of cavities per pod, and the number of seeds per pod.

- The well-watered soil regime treatment resulted in significantly ($p \leq 0.05$) highest number of trifoliate leaves, number of branches, chlorophyll content and stomatal conductance. Interestingly, the CV17B genotype fertilized at 90 kg P ha^{-1} had the highest leaf length and leaf area. Similarly, the CV17B genotype irrigated at 15 kPa and without P fertilization had a significantly higher stomata conductance.
- The P x M interaction effect revealed that the application of 30 kg P ha-1 under a well-watered regime (15 kPa) resulted in significantly ($p \leq 0.05$) the highest number of seeds per pod.

6.1.2 Results of laboratory determinations of plant tissues

- The obtained results revealed that all the main treatment factors evaluated had significant ($p \leq 0.05$) effects on the contents of cowpea grain protein, mineral and secondary metabolites except for genotype and P fertilization levels that showed inconsequential ($p \geq 0.05$) effect on the TSS and protein content.
- A significant increase in the TSS was observed under severed water stress (75 kPa) while the opposite in the case of protein content with a significantly ($p \leq 0.05$) reduced the protein content, suggesting a negative impact of soil moisture stress on protein quality. Moisture application at 15 kPa significantly increased flavonoid and phosphorus content in grain, while moisture stress imposition as 50 kPa significantly ($p \leq 0.05$) increased the content of anthocyanin, Fe and Zn remarkably. However, the CV18-1A genotype recorded the highest grain P and Zn content of 0.52% and 48.08 mg/kg respectively.
- The GxM interaction had inconsequential effect on TSS and protein content of cowpea grains. Similarly, the GxP and GxPxM interaction had inconsequential effect on the protein content. The CV17F genotype had about 10% higher flavonoid content than any of the tested genotypes while the control treatment without P addition produced cowpea grains with the significantly highest flavonoid content.
- Application of 60 kg P ha^{-1} resulted in substantial increase in grain P and Fe content. The CV17B genotype fertilized at 30 kg P ha^{-1} under a well-watered regime yielded

gave the highest TSS content. Similarly, CV17B genotype with 30 kg P ha^{-1} under severe moisture stress condition (i.e., 75 kPa irrigation) produced cowpea grains with highly elevated level of anthocyanin content. The CV17B genotype with 90 kg P ha^{-1} fertilization also under severe water stress resulted in a significantly reduced protein content. Furthermore, CV17I genotype with 60 kg P ha^{-1} under moderate soil water stress (50 kPa) produced cowpea grains with the highest (902.5 mg/kg) Fe content.

6.2 Conclusion and recommendations

The findings of this study revealed that genotype and moisture regime variation significantly impacted not only the cowpea growth, phenological and physiological attributes but the yield, and contents of grain protein, mineral and secondary metabolites as well as the WUE. Amongst all the tested genotypes, CV18-1A exhibited the greatest response in terms of growth, physiological and yield components as revealed in the highest mean number of leaves, plant height, chlorophyll content, pod length, number of cavities per pod, and number of seeds per pod. This was closely followed by CV17B genotype attributing to the longest leaf length, widest leaf area, highest stomatal conductance, highest mean number of hundreds seed weight, biomass at flowering, total biological yield and WUE at both flowering and harvesting. These unique attributes for the two genotypes represent critical factors for consideration in the selection criteria to optimize cowpea production as appropriate genotype selection by farmers will certainly promote greater yield and high return on investment. Moreover, the uniqueness of the two genotypes that can be further explored and applied through cowpea breeding programs for farmers' benefit include the ability to accumulate high concentration of mineral and secondary metabolites under low soil available P and severe soil moisture conditions thus impairing the effect of obesity and regulating major chronic disease such as diabetes, heart disease and stroke through consumption of the grain.

The results also revealed that the number of cavities in cowpea pods does not necessarily reflect the number of seeds, and the overall seed weight suggesting that possible poor grain filling due to moisture limitation. The latter is evident with CV18-1A, which recorded more seeds per pod but with much lower seed weight compared to CV17B, which has a small grain seed size and structure may influence the seed yield and economic return (income) for farmers. Although P fertilization did not necessarily result in yield increases, a positive and significant response was observed on biomass production and WUE during the transition from vegetative to flowering stage. This is crucial for farmers who may wish to harvest fresh cowpea leaves as leafy vegetable locally consumed as "*morogo*". Moreover, P fertilization also promoted an increase in the overall nutrition (i.e., mineral and secondary metabolite content) of the cowpea grains except for the protein and TSS contents. Based on the results obtained, the hypothesis that the growth, yield, and nutritional parameters of the four cowpea genotypes will not differ under variable soil available P and moisture regimes is hereby rejected. Similarly, the optimum P rate for the four cowpea genotypes differed remarkably based on each measured parameter. Furthermore, WUE and yield of each cowpea genotype differed across the varying soil moisture levels. The results underscore the potential use of integrated agronomic strategies of 60 kg P ha^{-1} under moderate (50 kPa) irrigation regime to enhance cowpea productivity and profitability by looking at the food and nutrition security situation in South Africa, particularly the limited availability of indigenous foods in the country's food market. Although the crops can tolerate moisture stress conditions, optimizing yield and grain quality is still dependent on access to minimal irrigation supplements. Therefore, the study proposes the adoption of an improved P and moisture management strategy with proper selection of genotypes for effective cowpea productivity and maintaining grain quality (i.e., nutrition and secondary metabolites).

6.3 Possible future research works

- Field-validated empirical data, possibly across various soil types and agro-ecological zones, is required to complement the current greenhouse-based data for sound and reliable recommendations to inform management decisions to promote cowpea production.
- The screening of P and moisture stress effects on the inflammatory properties of cowpea grains to reduce bloating, promote the repair of damaged tissue, and restore homeostasis can be explored for a holistic recommendation. This should also include reducing the content of oligosaccharide and trypsin inhibitors (e.g., tannin), which are major factors that have been reported to limit cowpea consumption.
- There is a need to introduce phosphate solubilizing microorganisms in future studies to assess if the organism cannot enhance P uptake and minimize P fixation.